X_{Cm} | BcII | Hin dIII | Eco RI FspI'IR AmpR Eagl FLP ori XbaI Aval pSAC35 11037 bps IR NotI Eag I REP2 ori Xbal, Fspl Xcml, IR LEU2 REP1 Eco RI Xcm I Hin dIII FspIEcoRI, Hin dIII Aval PstIHpa1

Figure 2

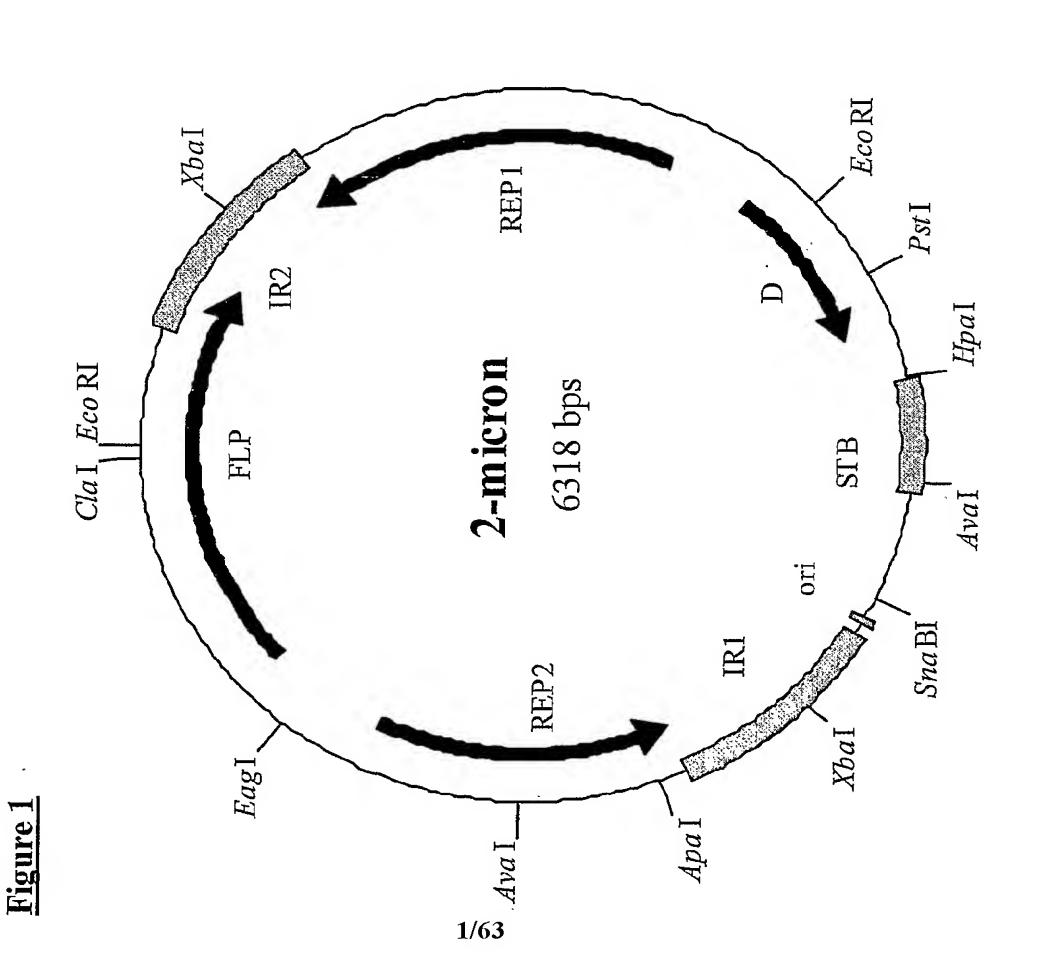


Figure 3

Insertions in FLP and the FLP Inverted A) Restriction Endonuclease Sites used for DNA

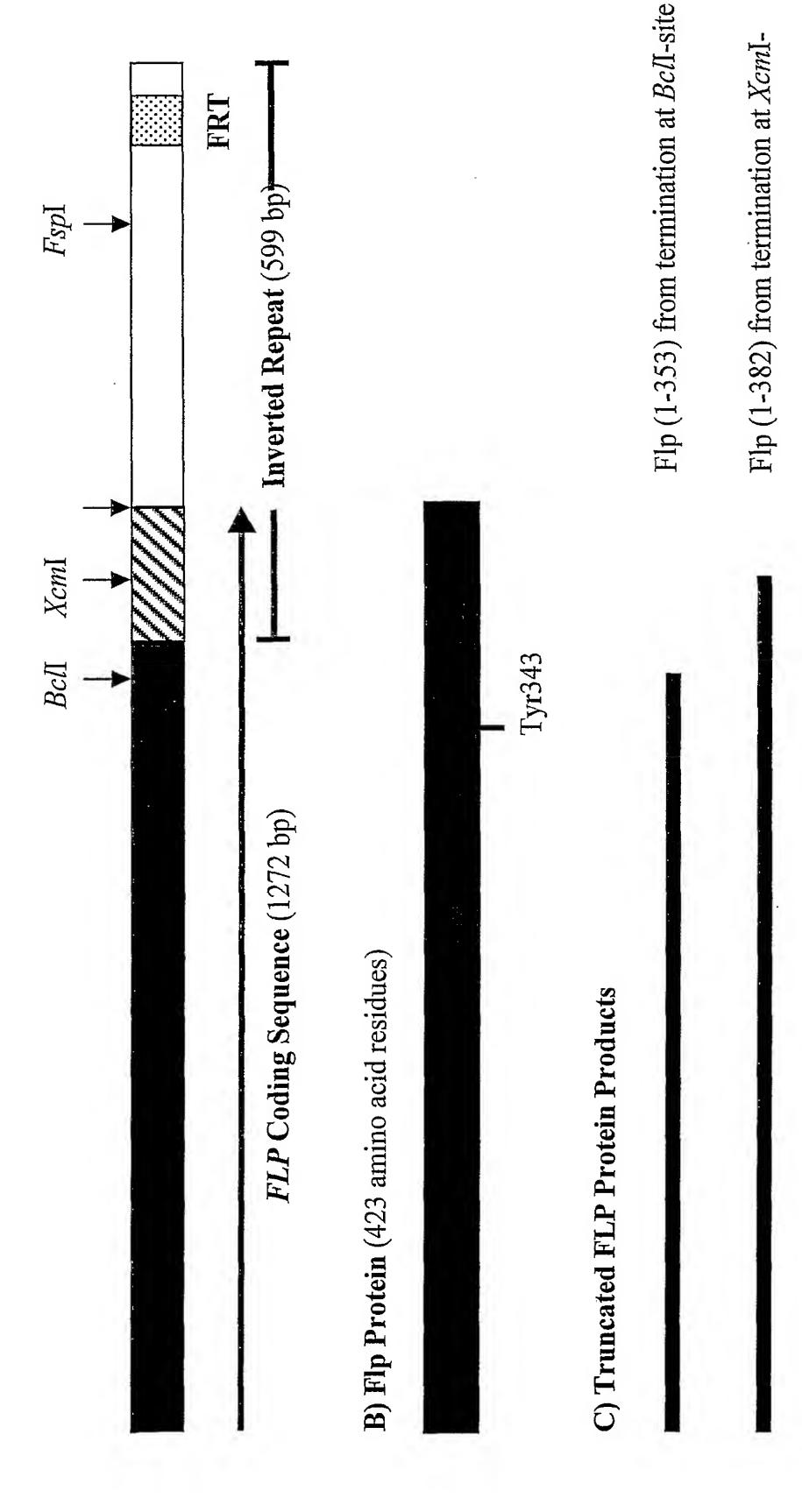
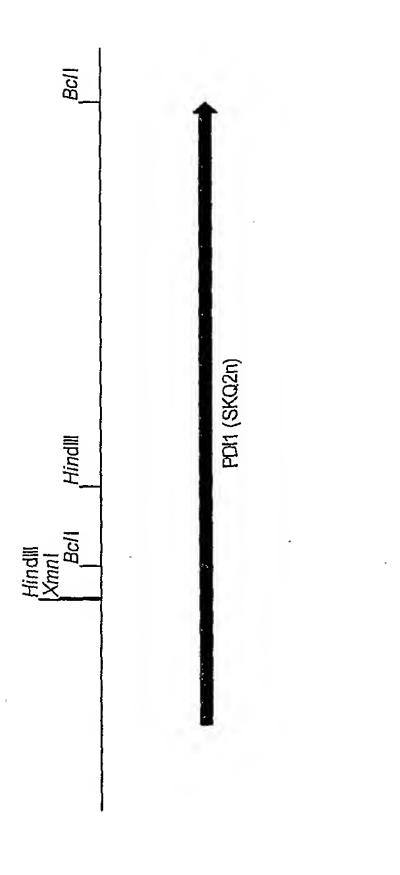


Figure 5

Figure 4



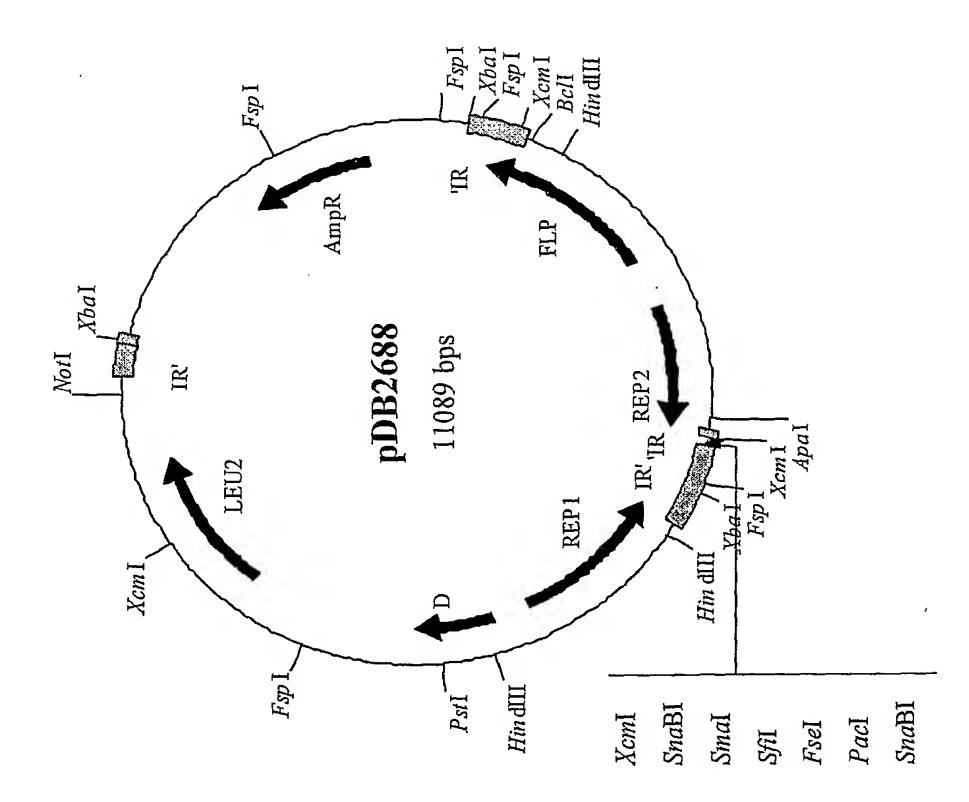
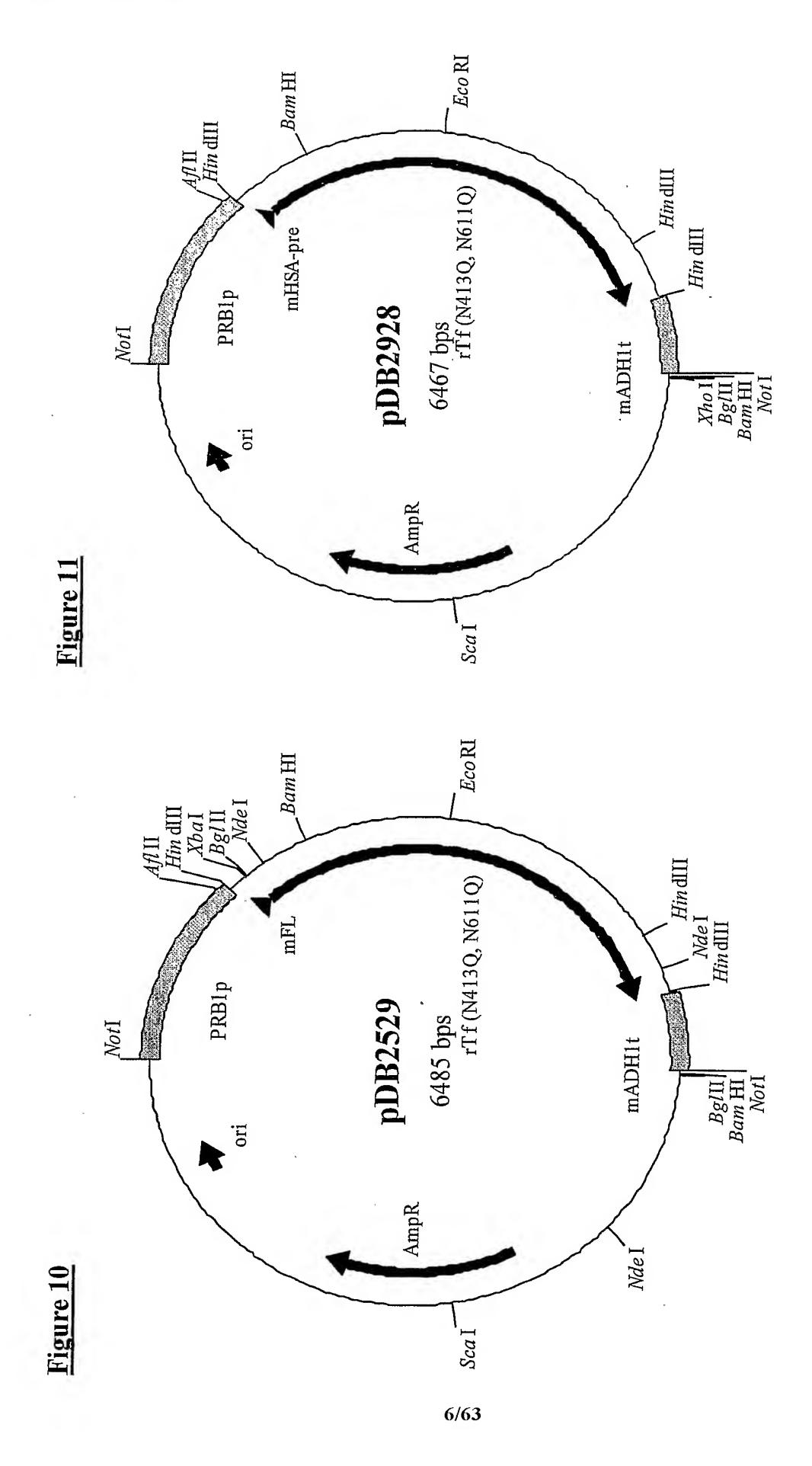
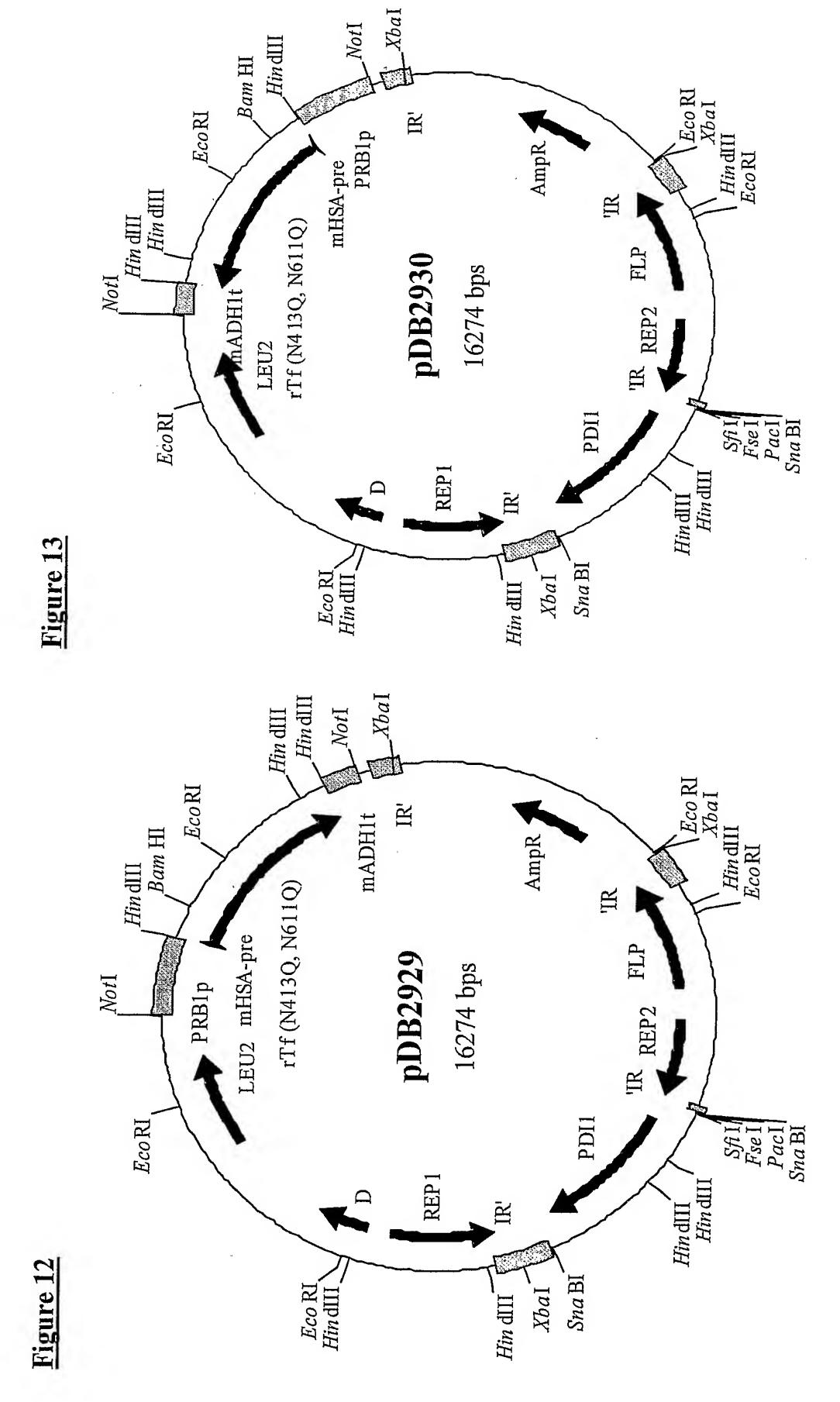


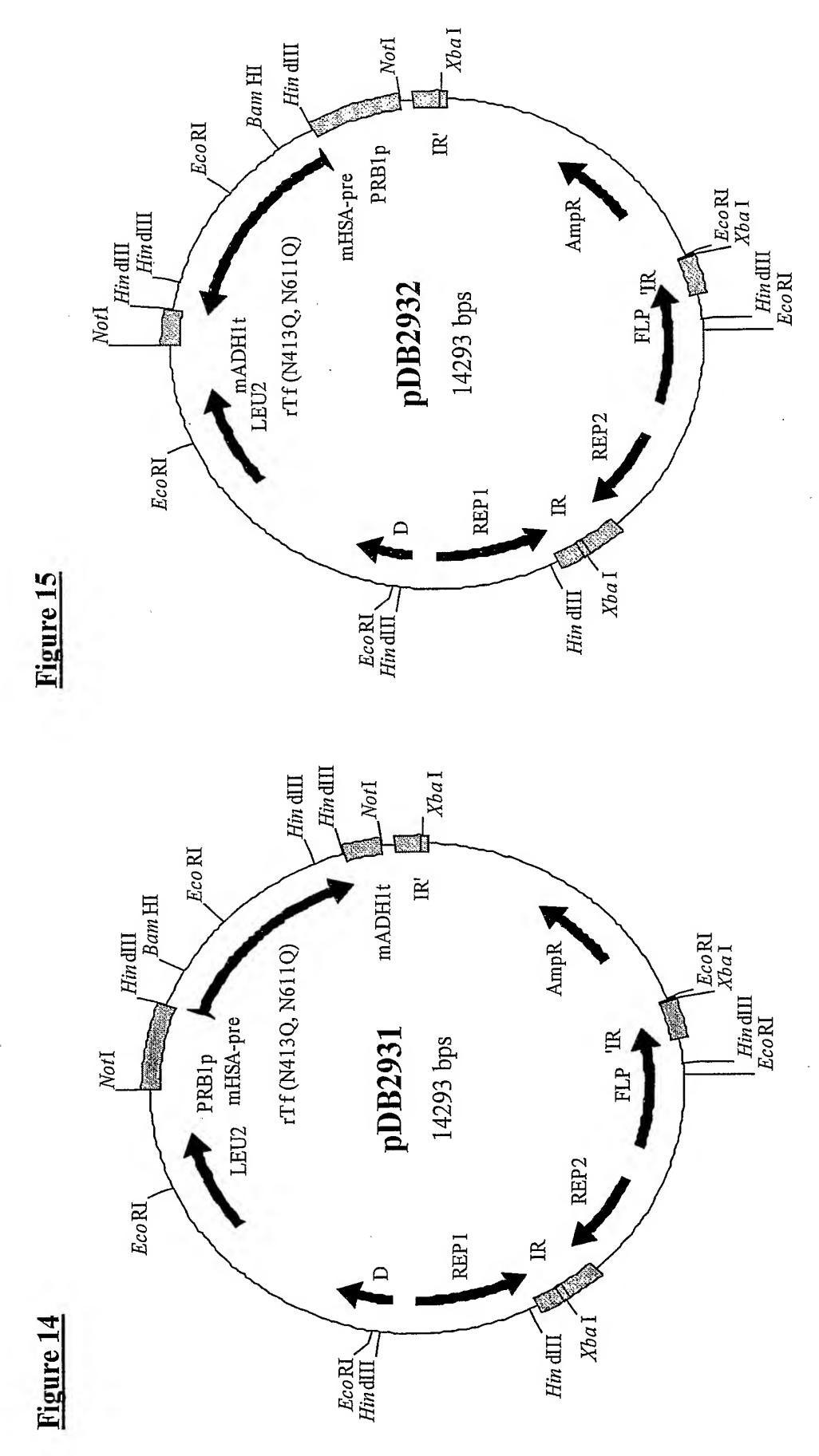
Figure 7

mFL = modified HSA(pre)/MF α 1(pro) fusion leader sequence XbaIHin dIII Sph I NotI Hin dIII Nde I PstI StuI $\bigwedge_{\substack{Nde \ I\\ Nde \ I\\ BclI}} Nde I$ IR' $(A_{BgIII}^{Indl.})$ mADH1t AmpR . Modified Tf PstI IR. PRB1p 7 SphI FLP 16292 bps pDB2711 NotI REP2 IR Sfil Fse I Pac I Apa I PDI1 REP1 Hin dIII / BcII / Hin dIIIIR Bcl1 / Stu1 BgIII PstI Stu I Hin dIII Xbal Hin dIII Stu I XcmI BcII HindIIIFsp I Xba I Fsp I Fsp I FLP AmpR | Apal |Xcm | |Sna Bi |Pac | |Fse I |Sfi | REP2 XbaI |HindIII| BcIITR. pDB2690 13018 bps NotI IR' PDI1 HindIII LEU2 R XcmI REP1 Xba I / Fsp I / Xcm I / Sna BI , BcII FspI Hin dIII Figure 6 PstI Hin dIII

Eco RI NdeIAff II Hin dIII AccI mHSA-pre = modified HSA-pre leader sequence Stul rTf (N413Q, N611Q) mHSA-pre Hin dIII PRB1p Acc I Hin dIII Bam HI pDB2921 5858 bps lac AmpR Figure 9 Scal mFL = modified HSA(pre)/MF α 1(pro) fusion leader sequence Bam HI EcoRI AfIII Hin dIII XbaI BgIIIModified Tf mFL Hin dIII PRB1p pDB2515 5876 bps lac Bam HII Hin dIIIAmpR Figure 8 Xmn I Scal







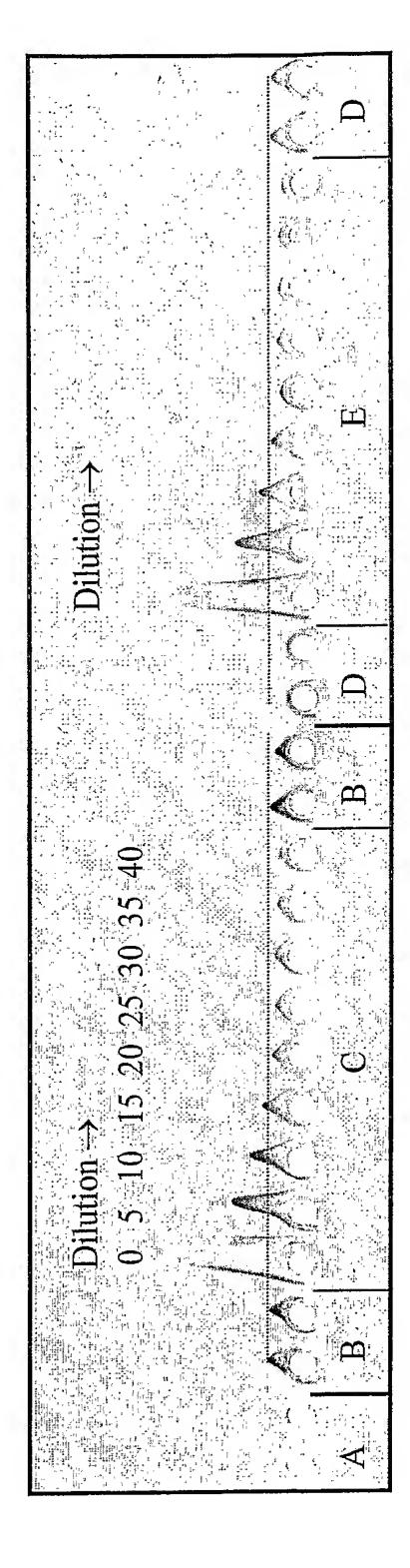
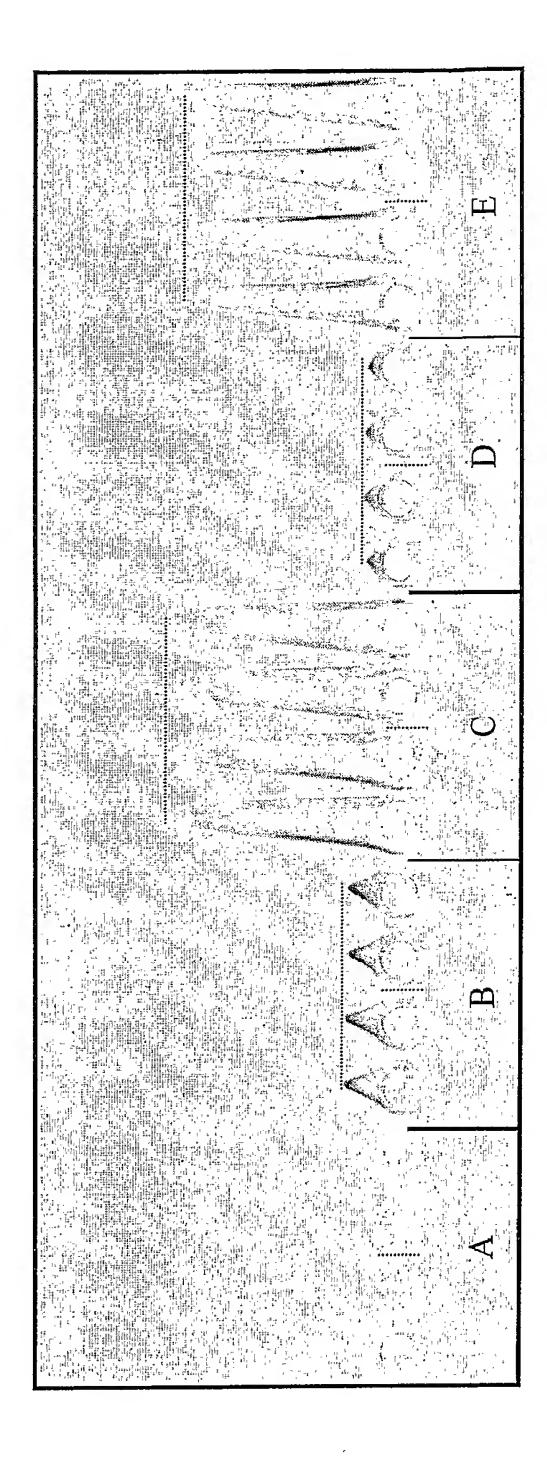
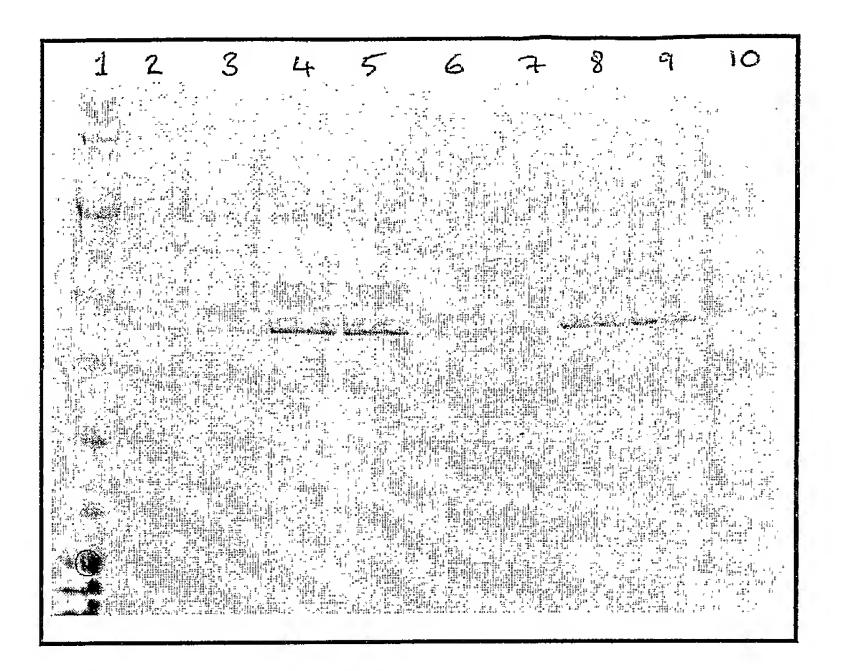


Figure 16



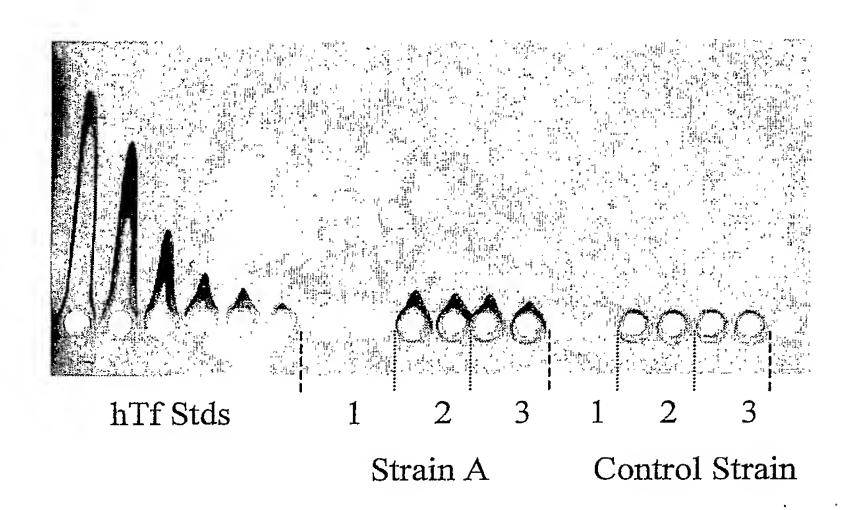
igure 17

Figure 18



5

Figure 19



5

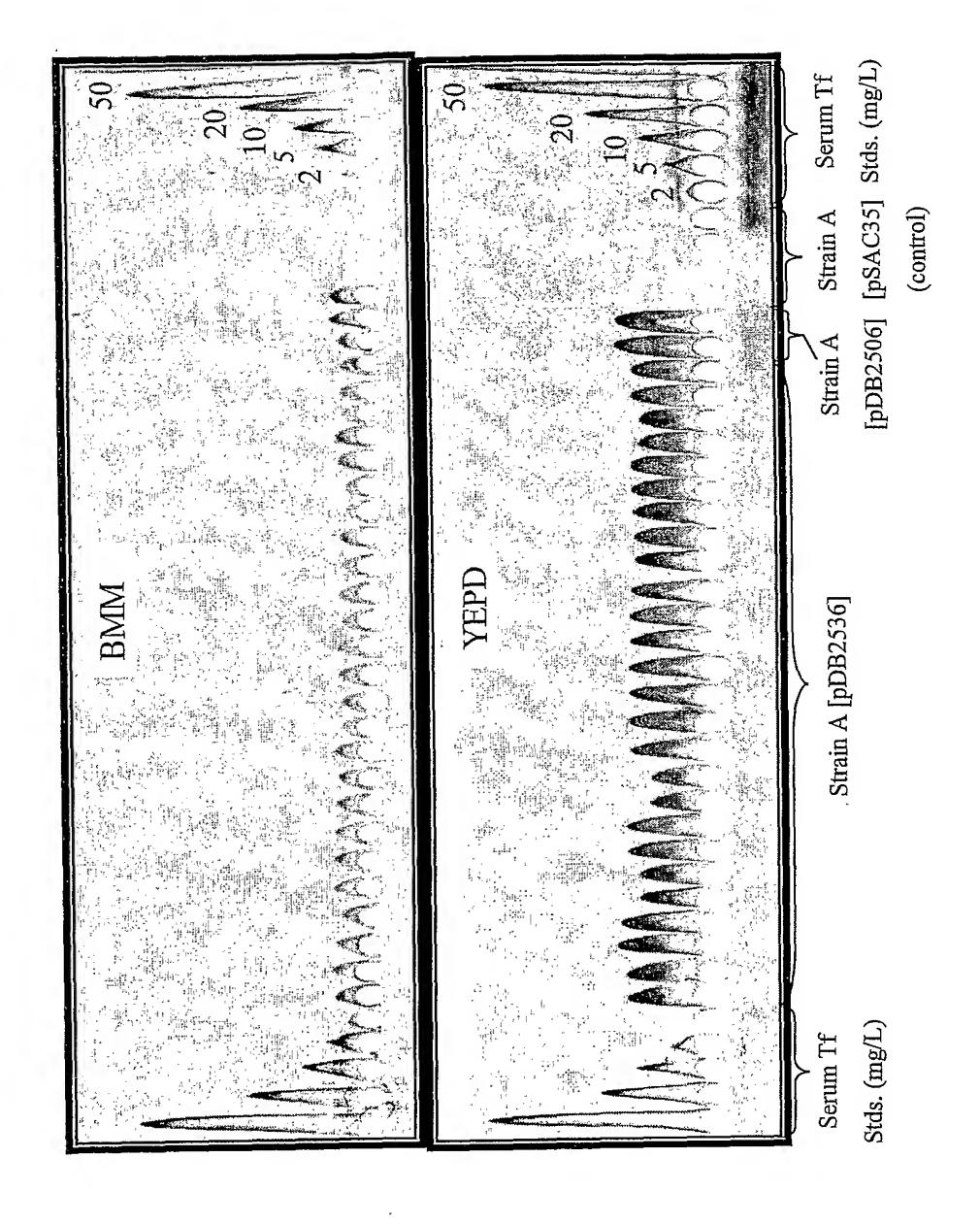


Figure 21

1 2 3 4 5 ≈ Mol. Wt.

5

191kD

97kDa

64kDa

51kDa

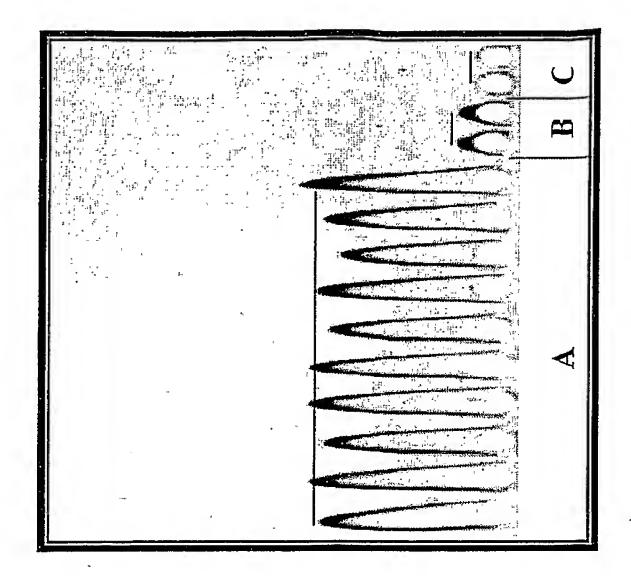
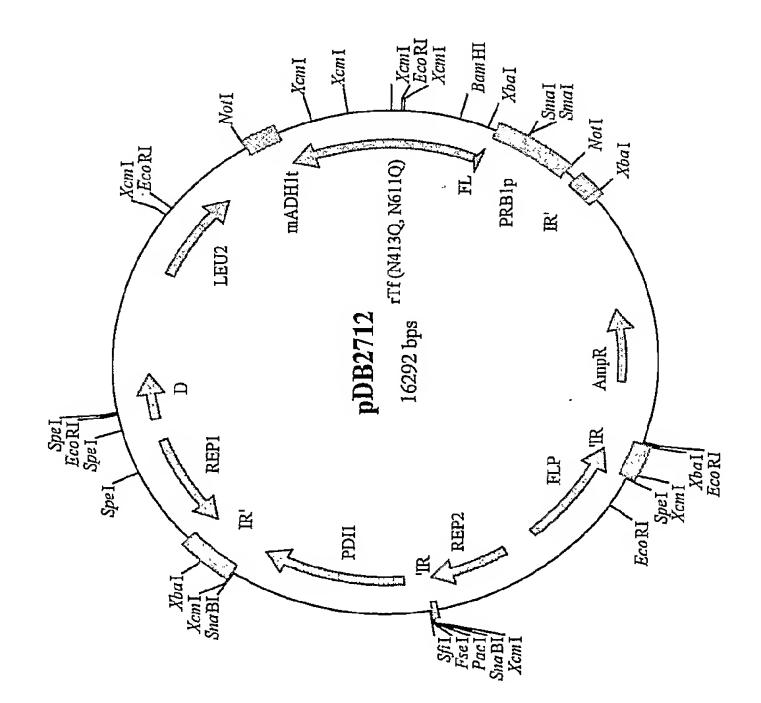
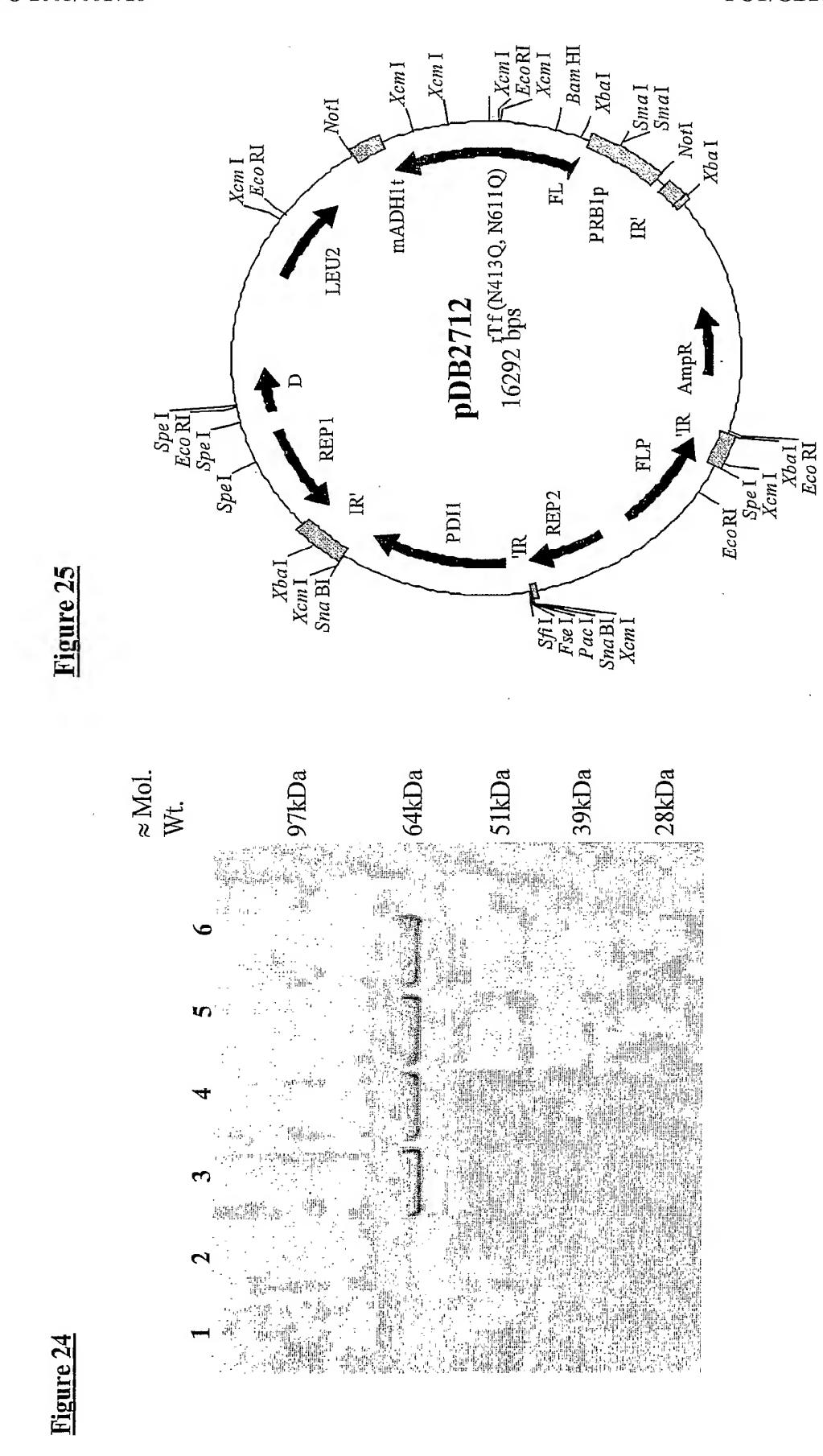
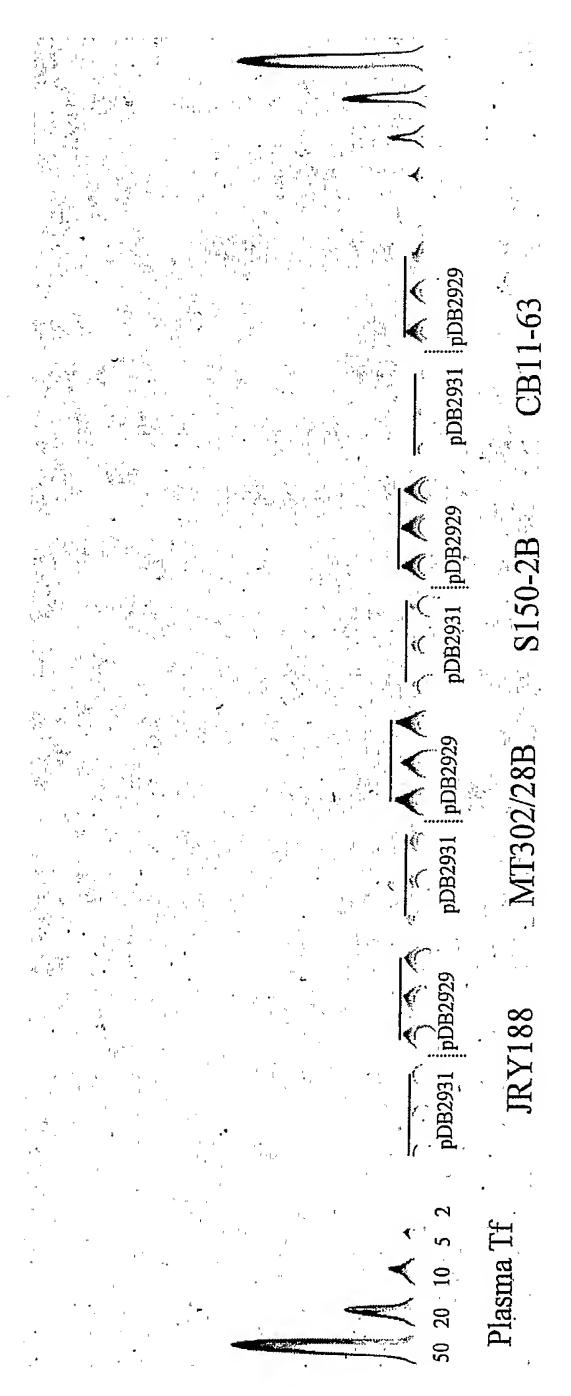


Figure 23







ligure 20

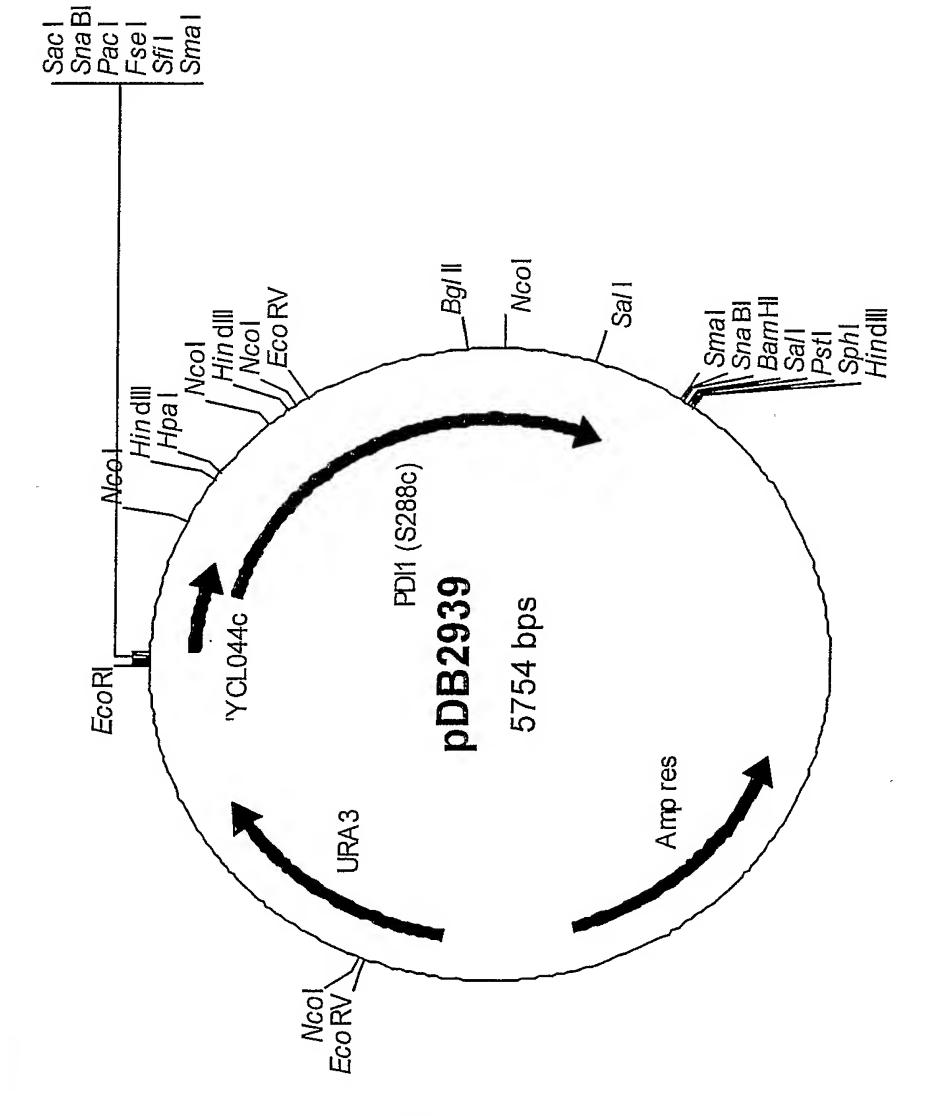
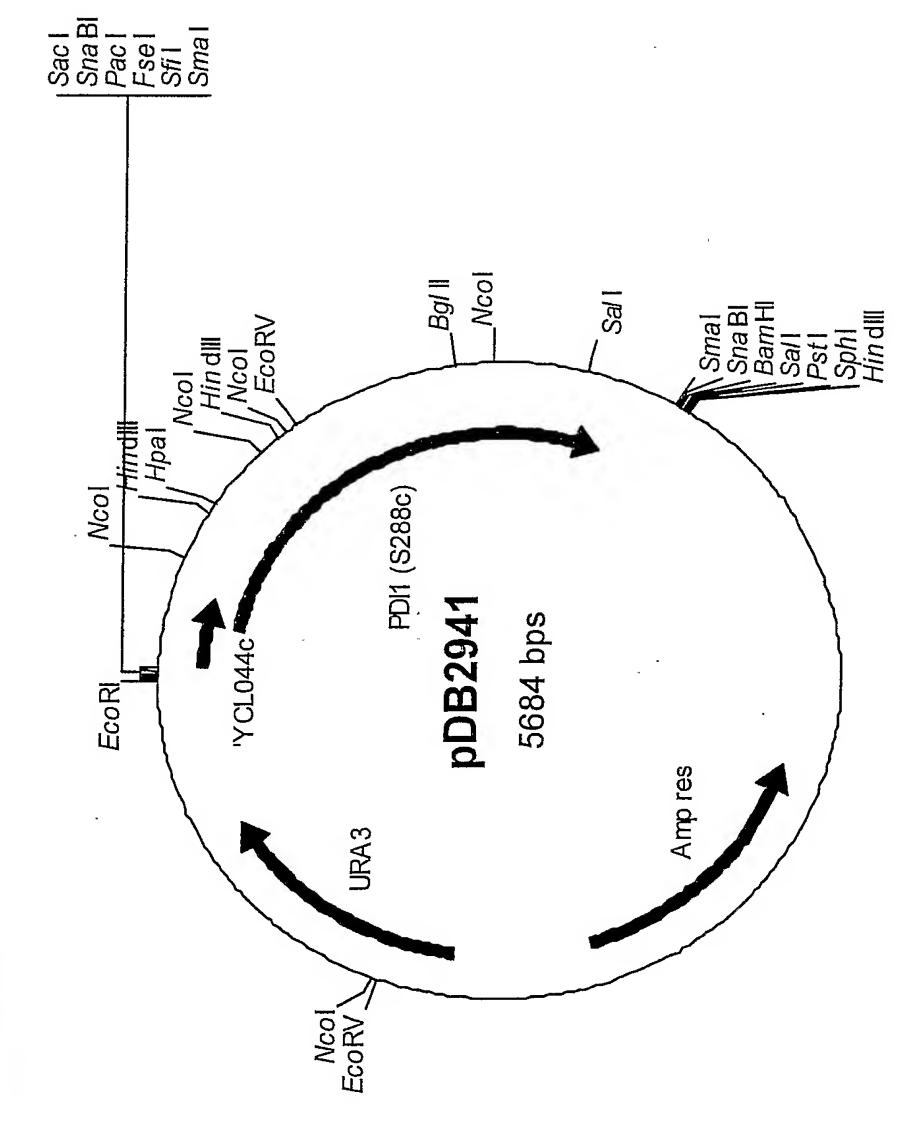


Figure 27



ligure 28

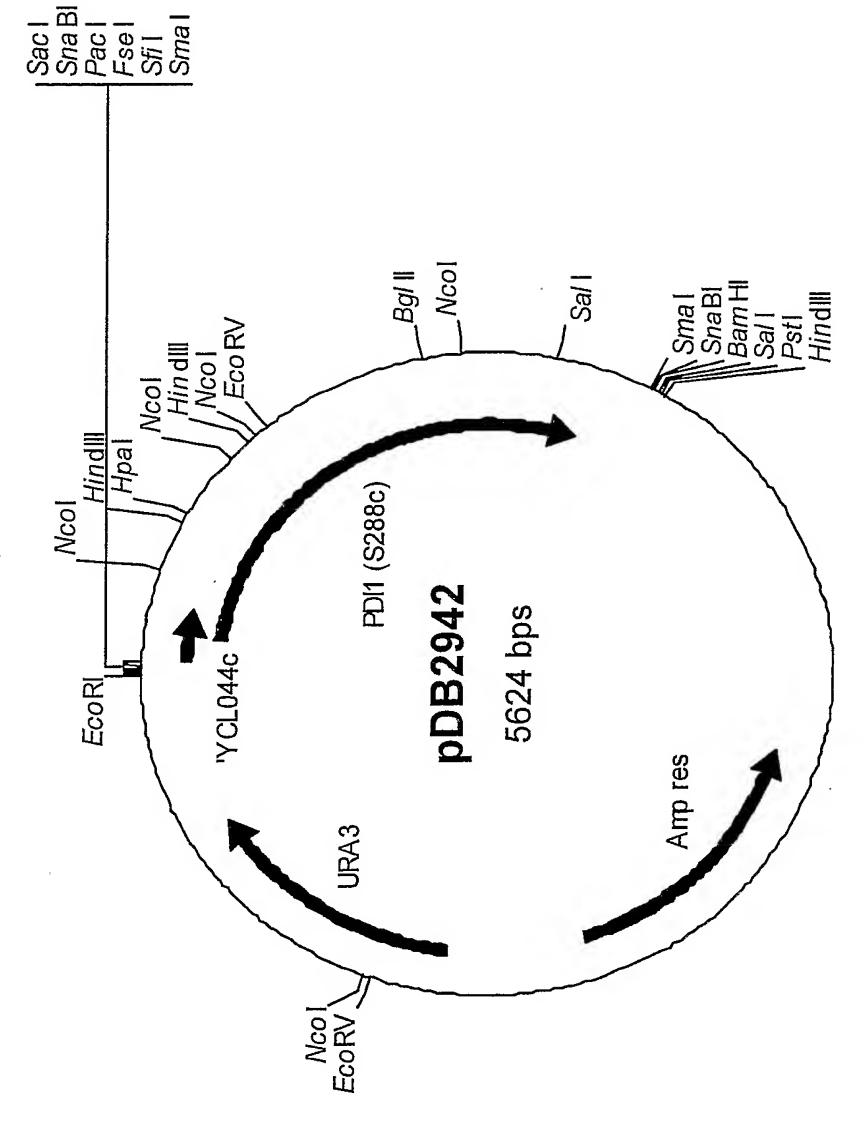


Figure 29

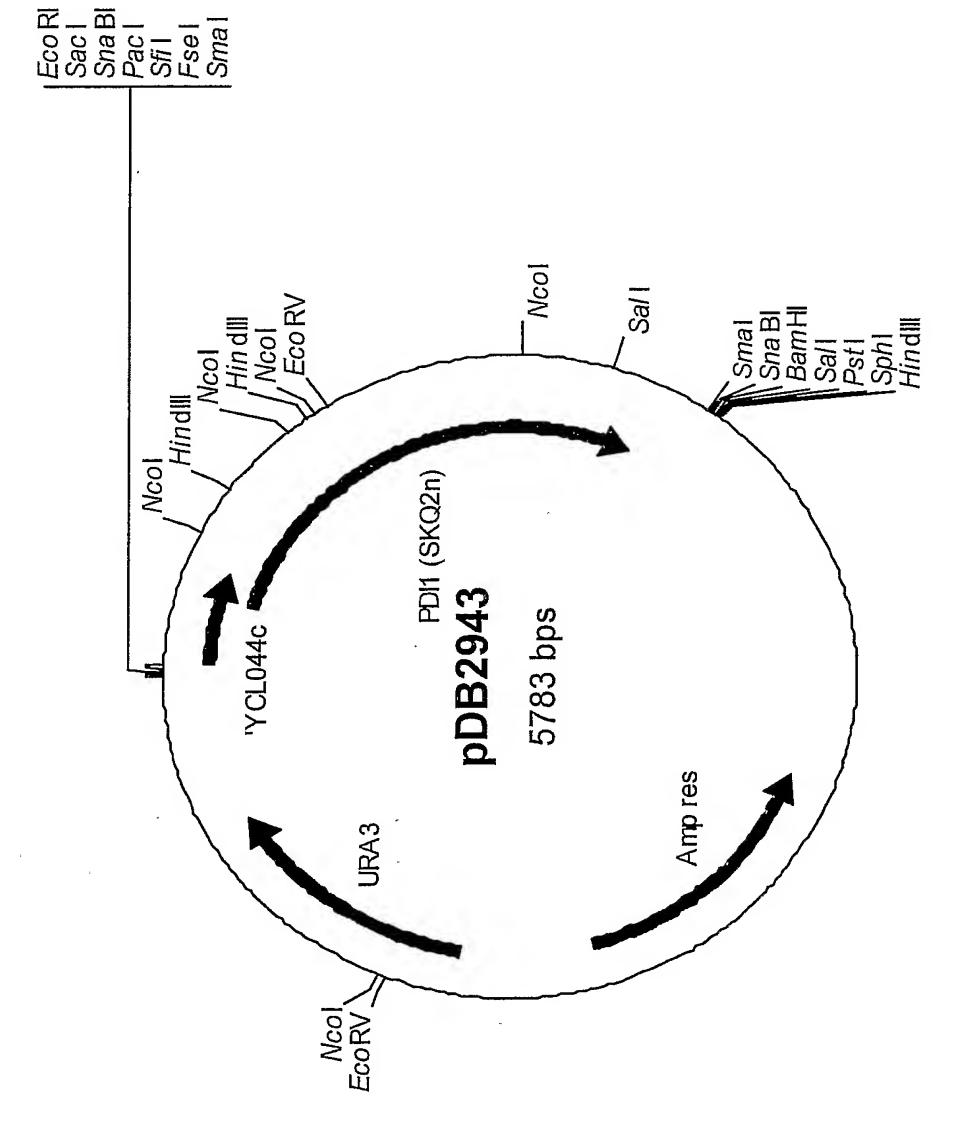
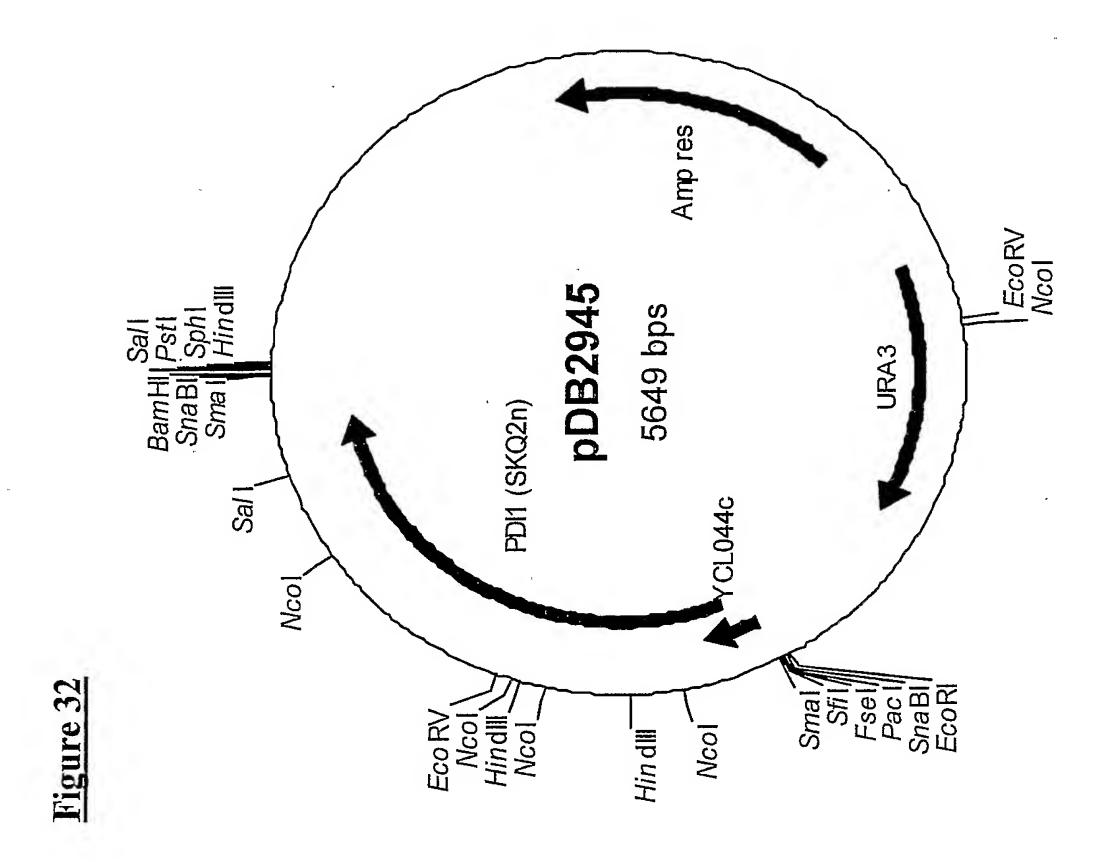
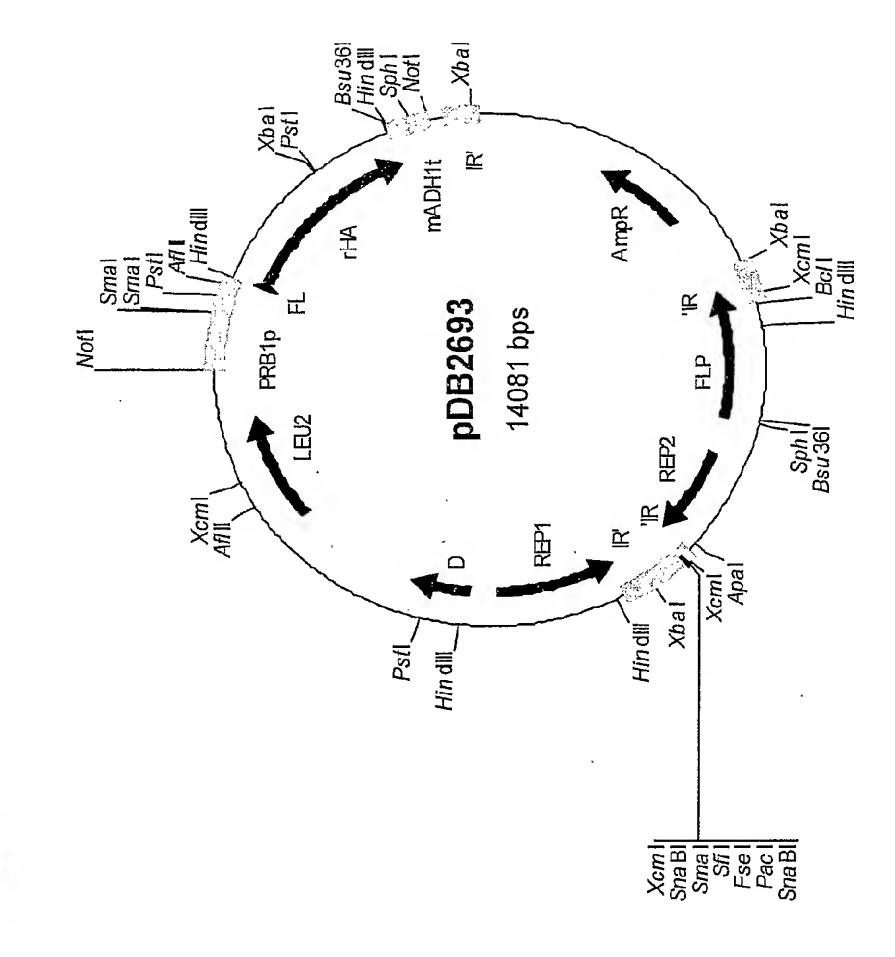


Figure 30



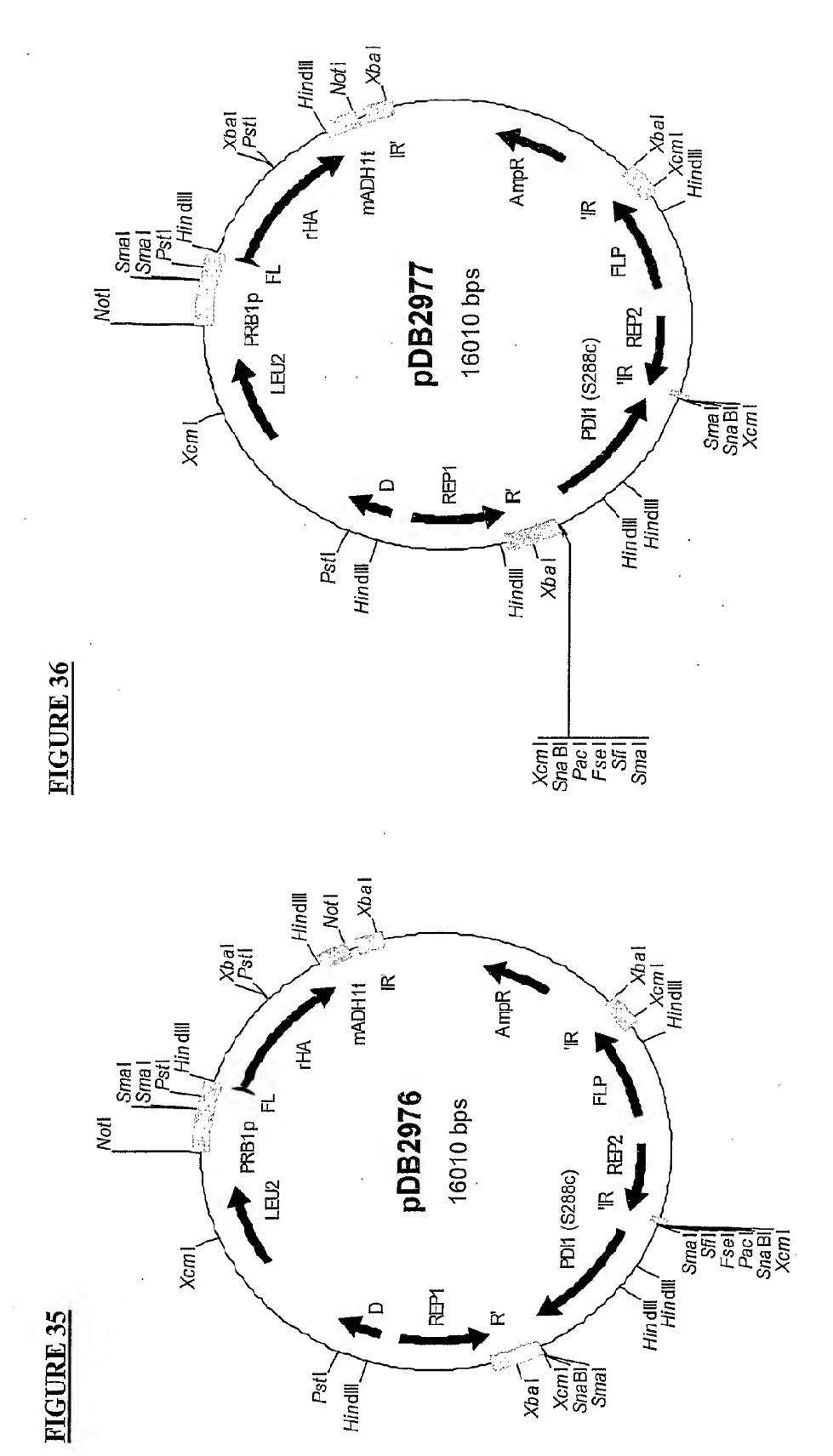
EcoRV
Ncol
Hin dill
Small
Ncol
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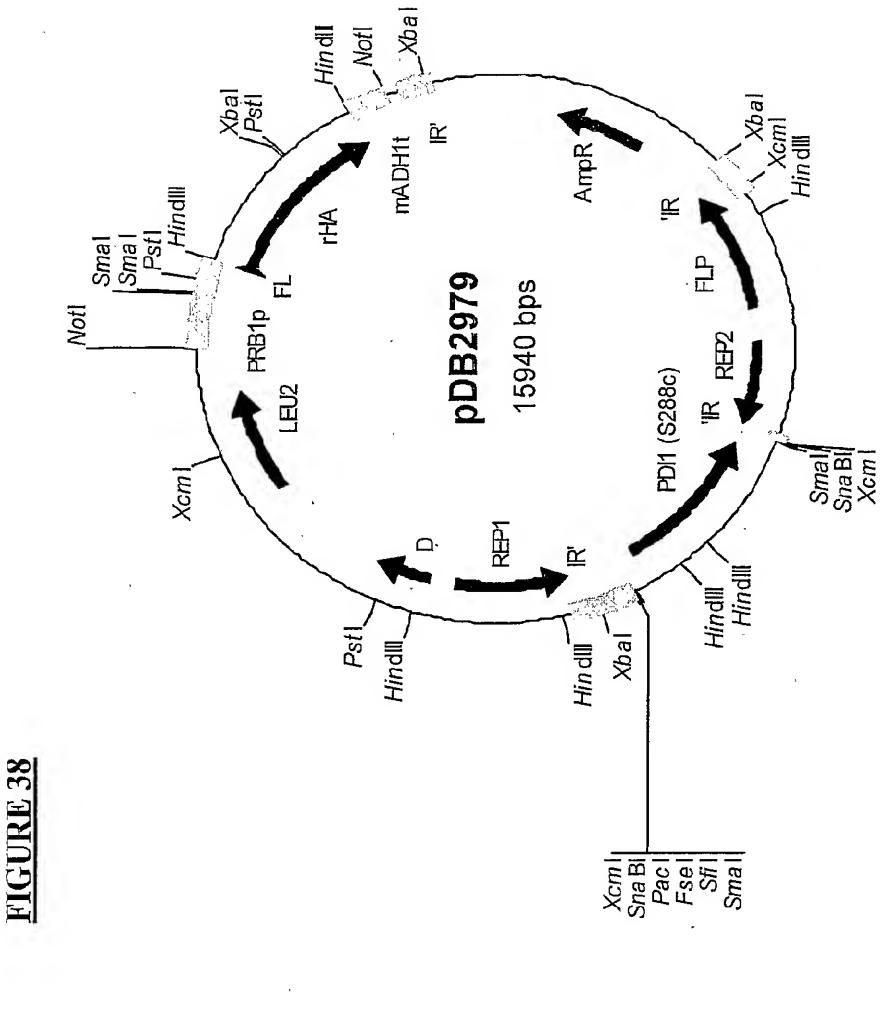
Figure 31



Ncol Xbal Aff III Affil Hindill rHA , HindIII 교 Nael PRB1p mADH1t pDB2243 6203 bps Notl **A** Nael Afi≡ AmpR Figure 33

Figure 34





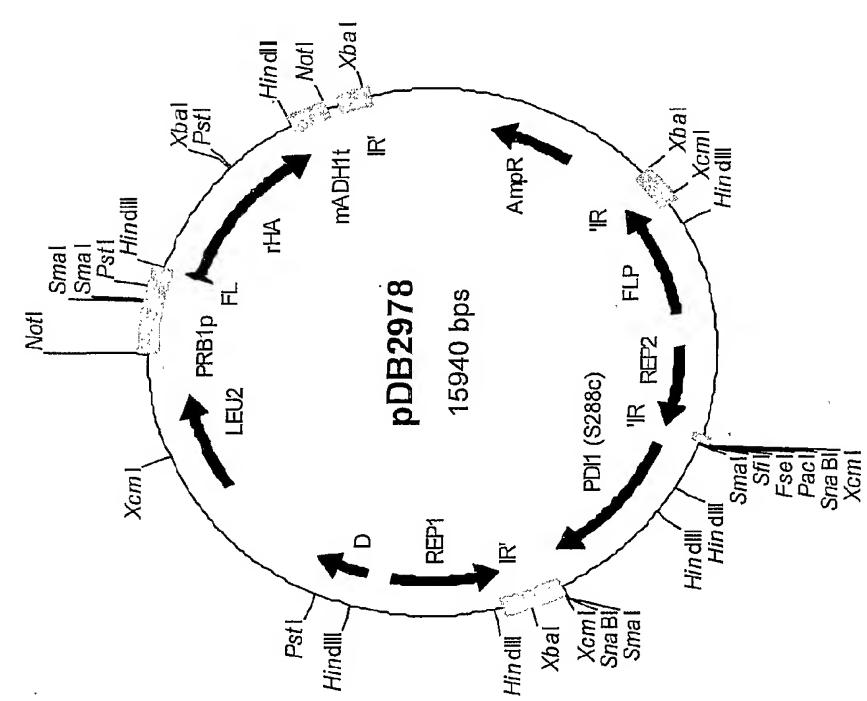
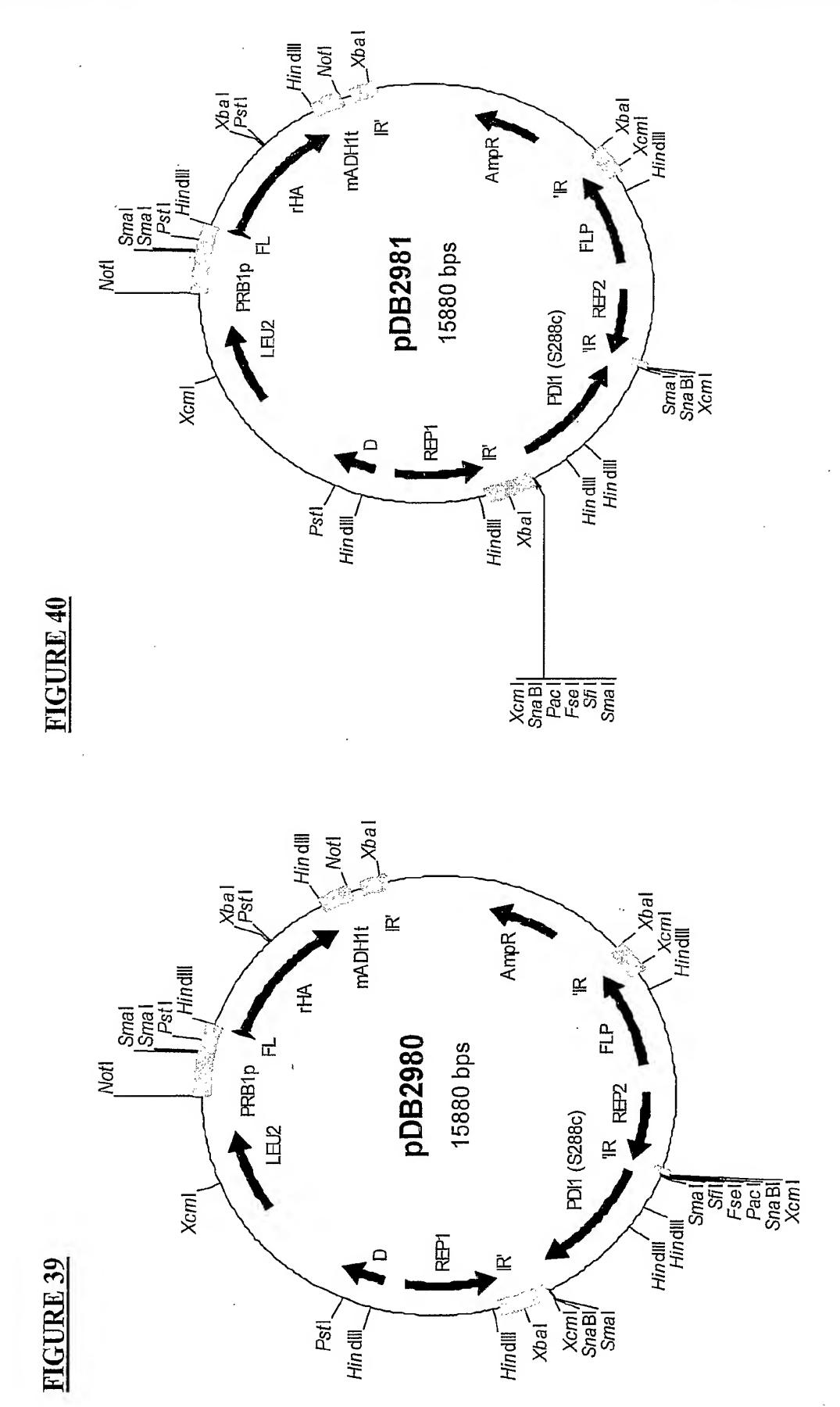
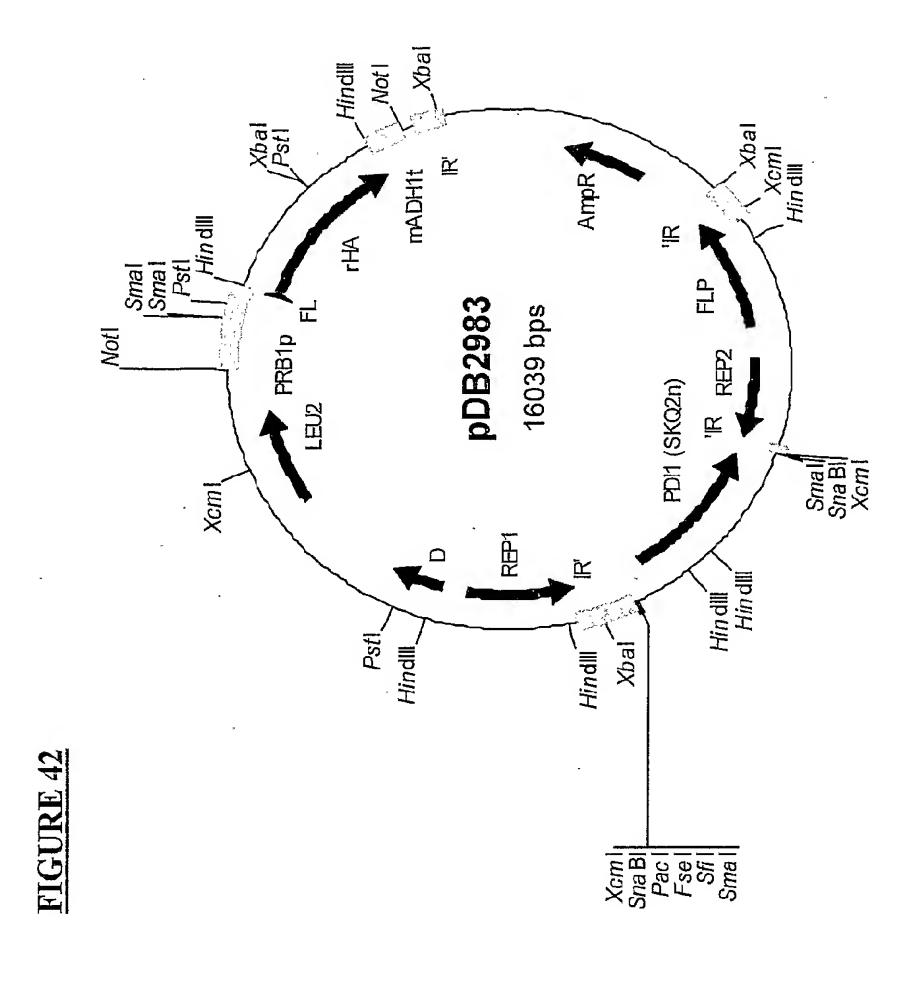
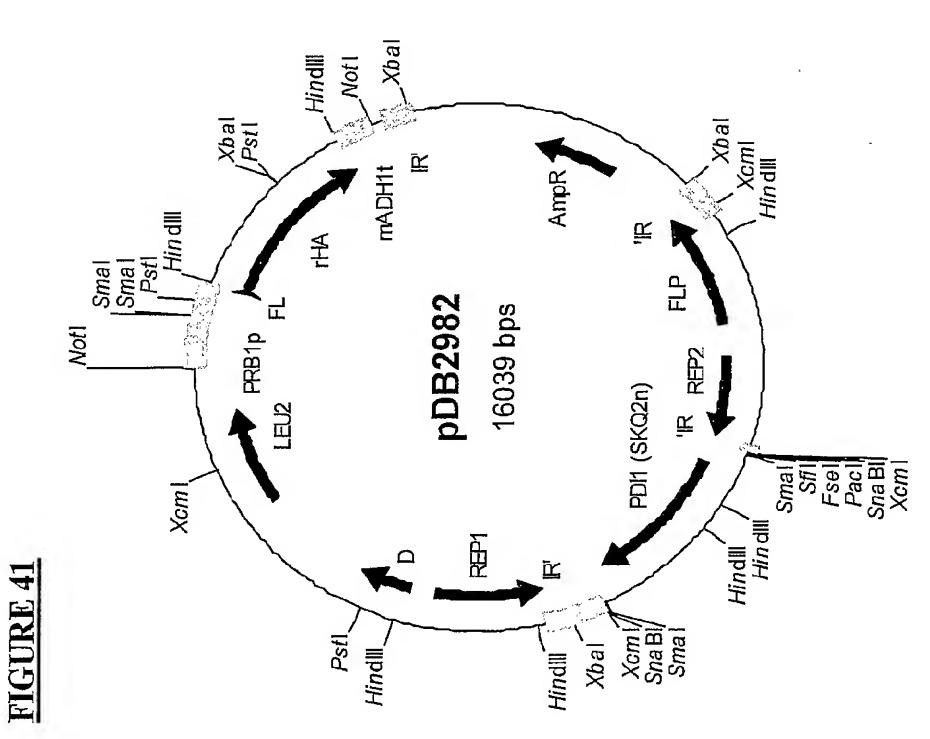
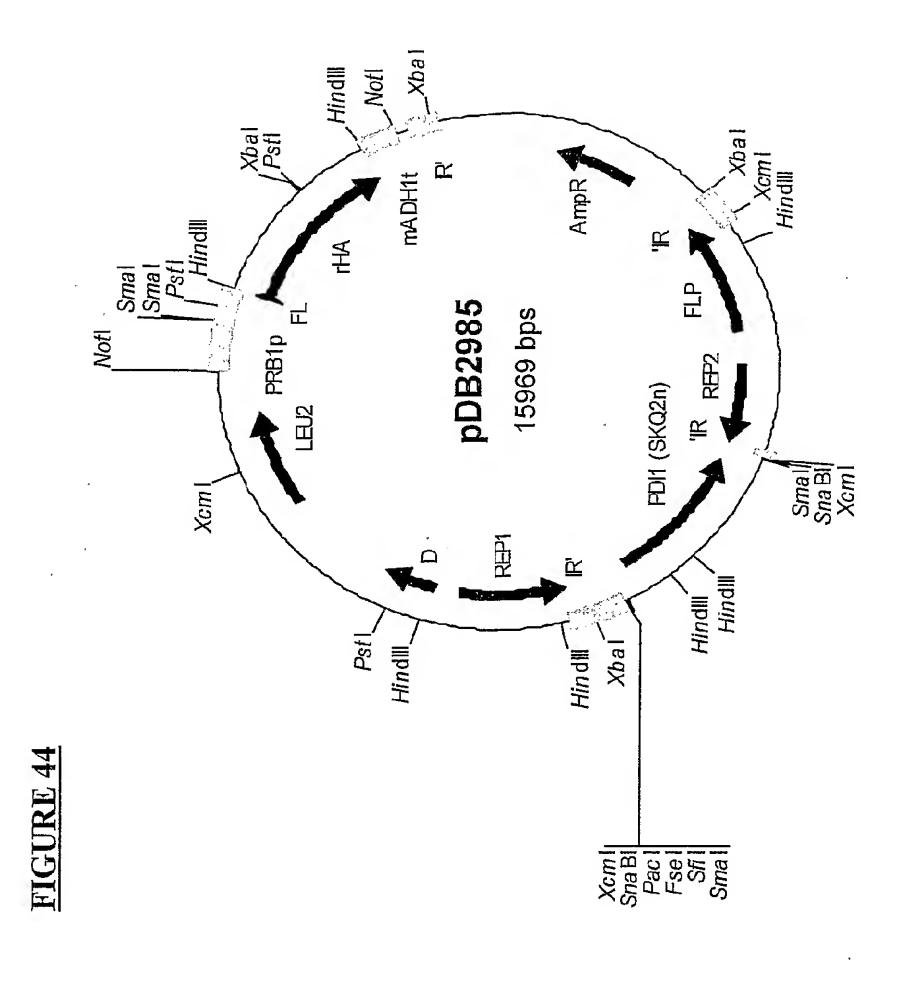


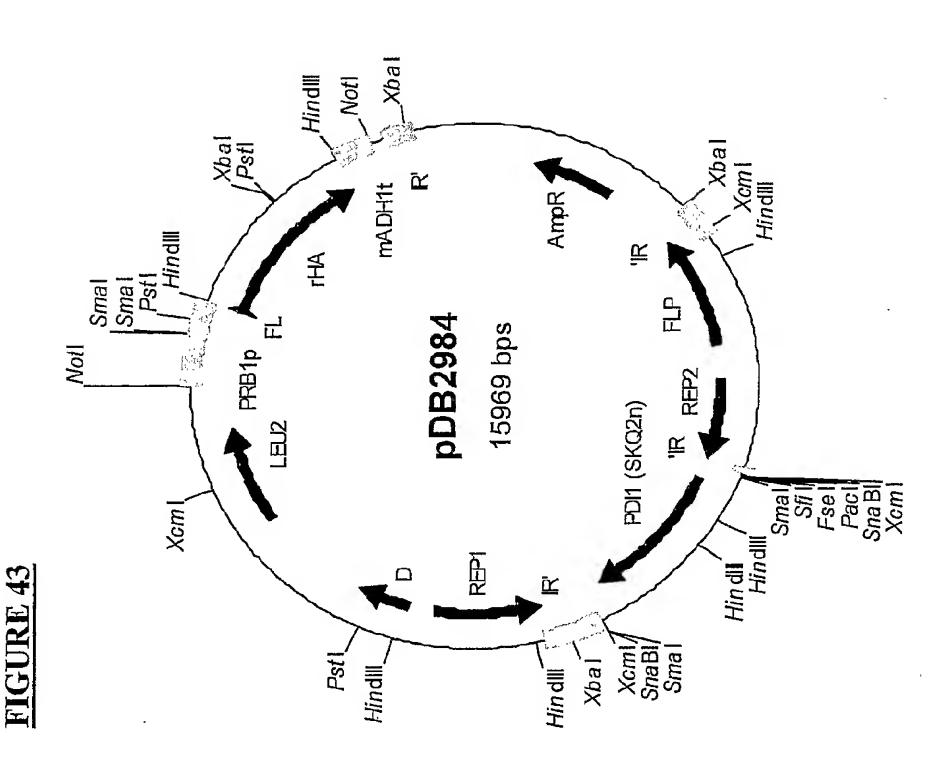
FIGURE 37

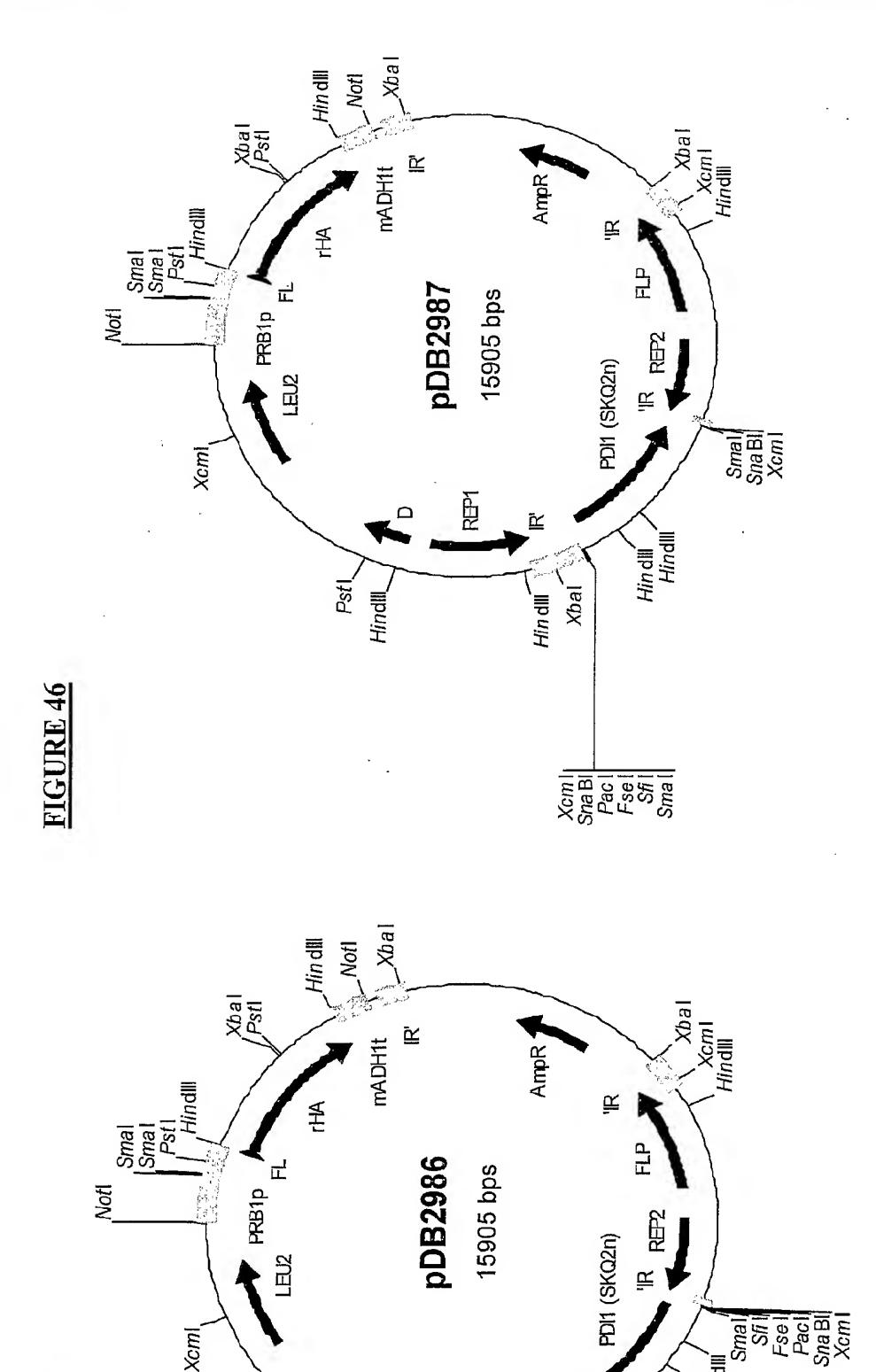












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HindIII

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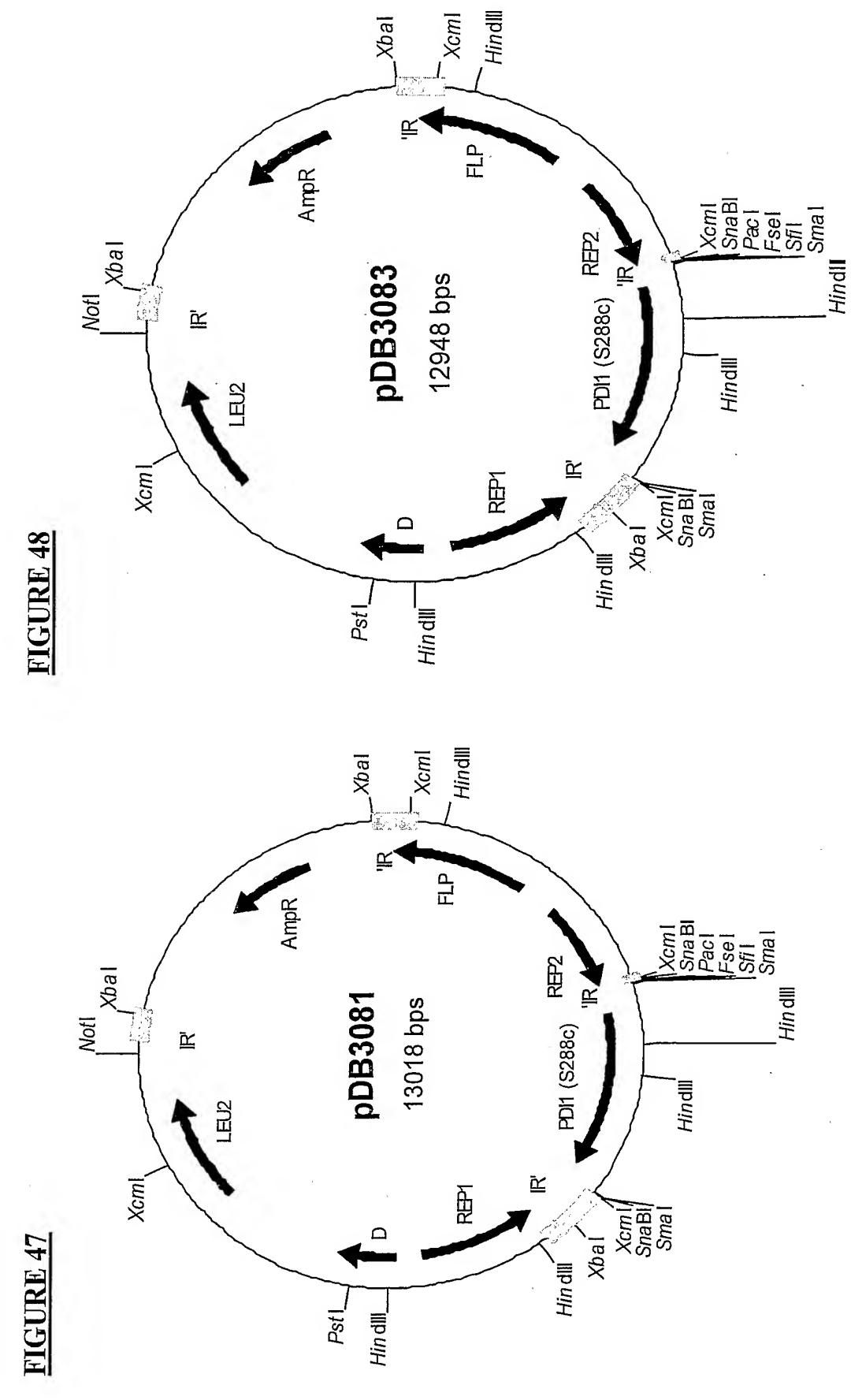
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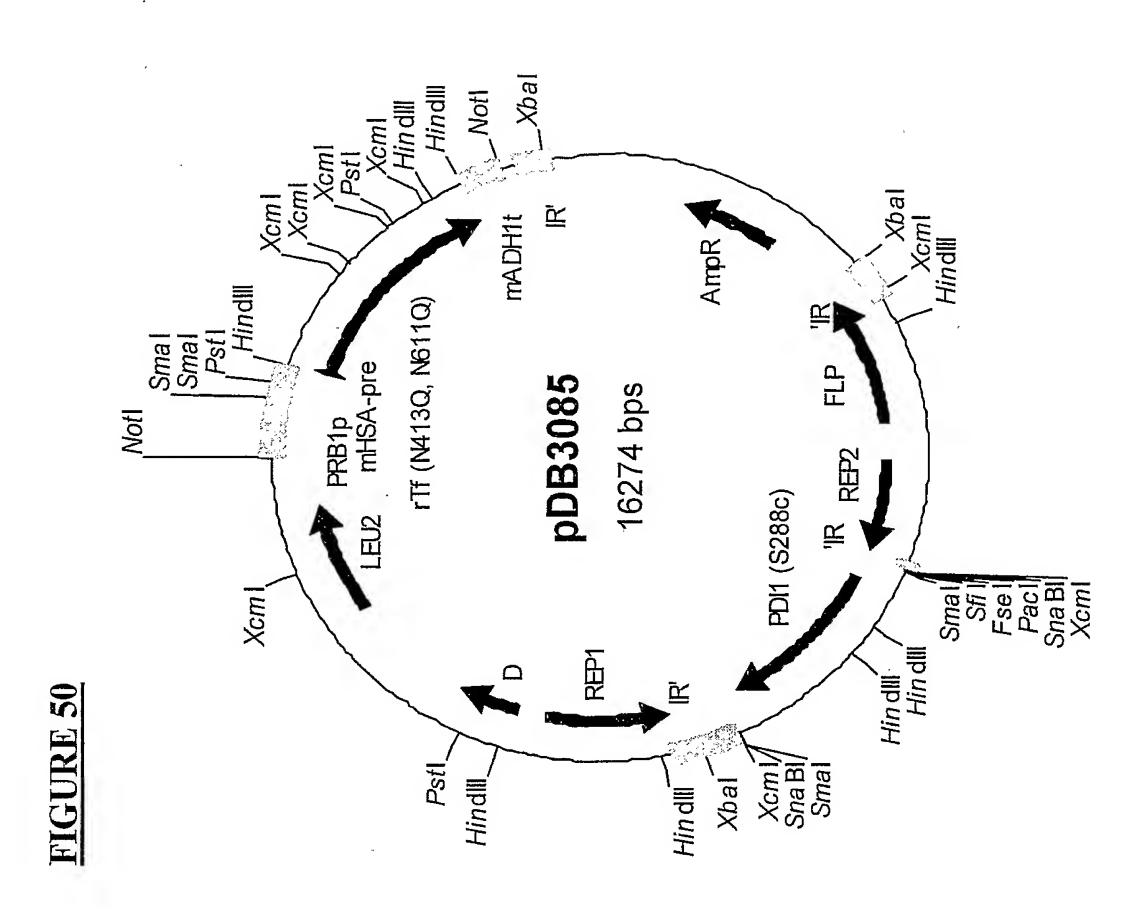
HindIII HindIII

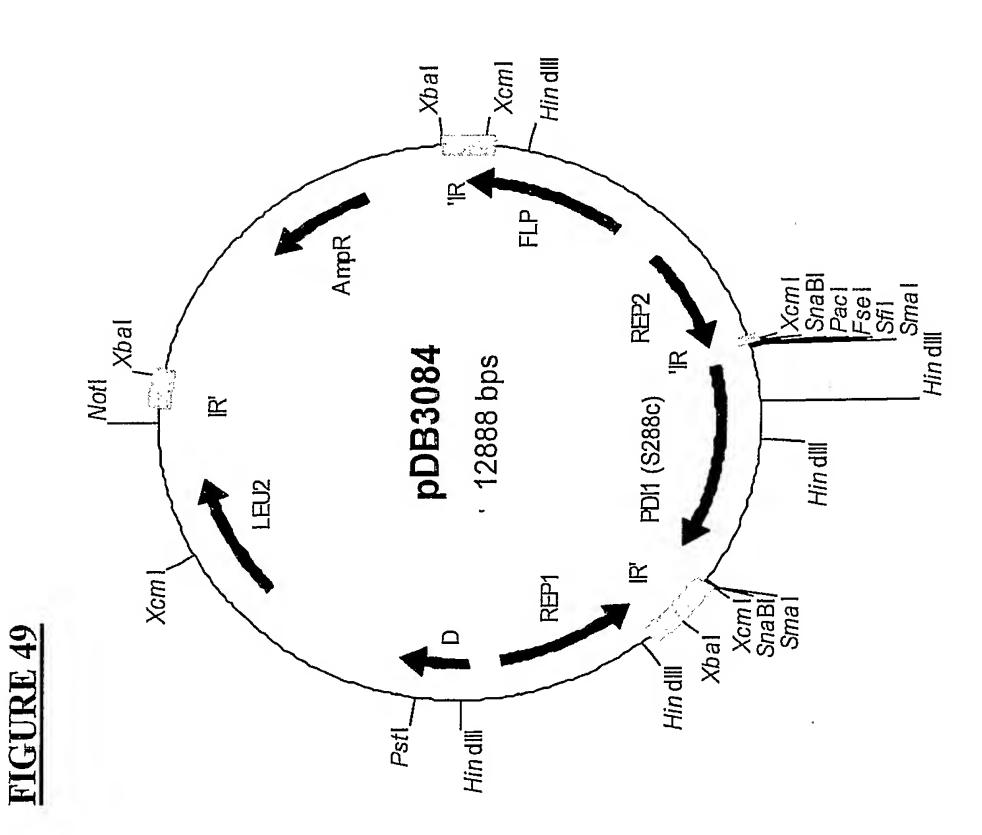
Xcm [/ Sna Bi/ Sma l

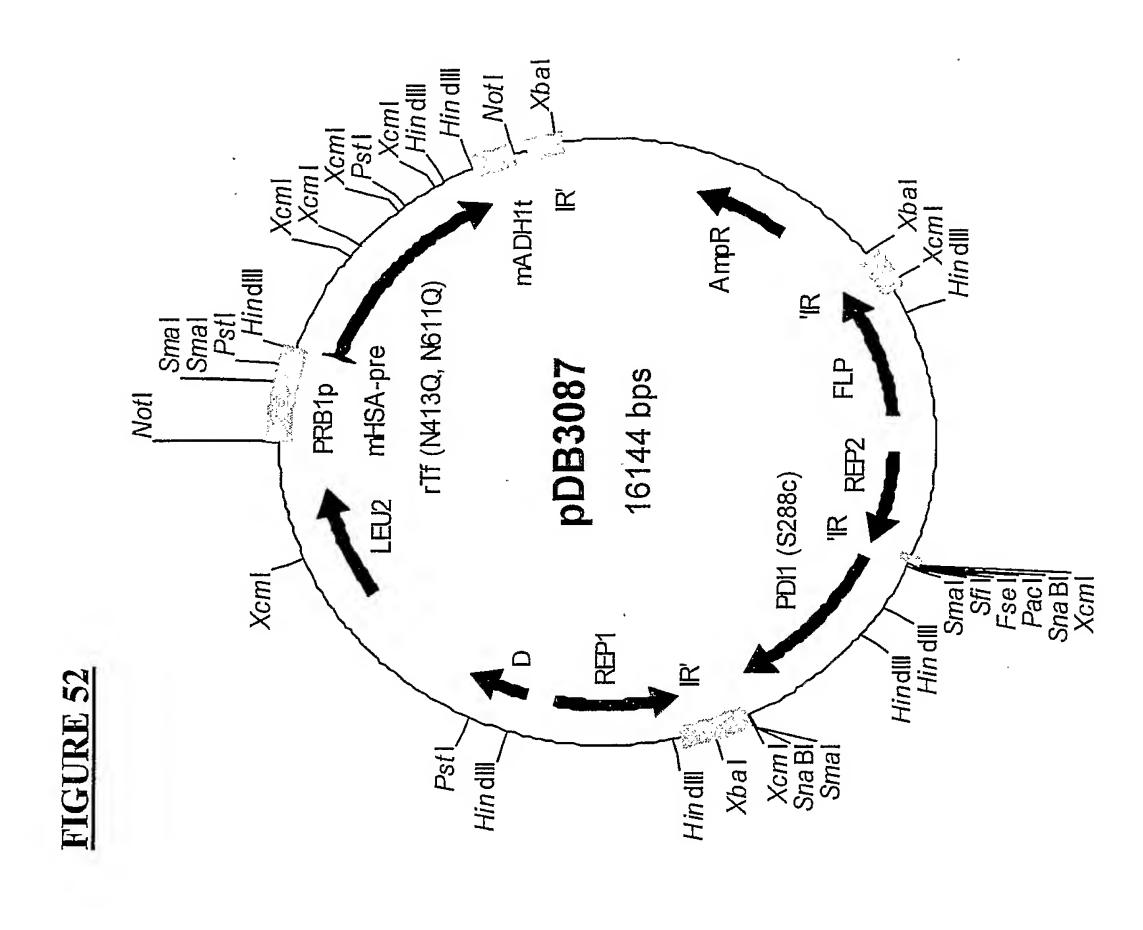
Xbal

Xcml





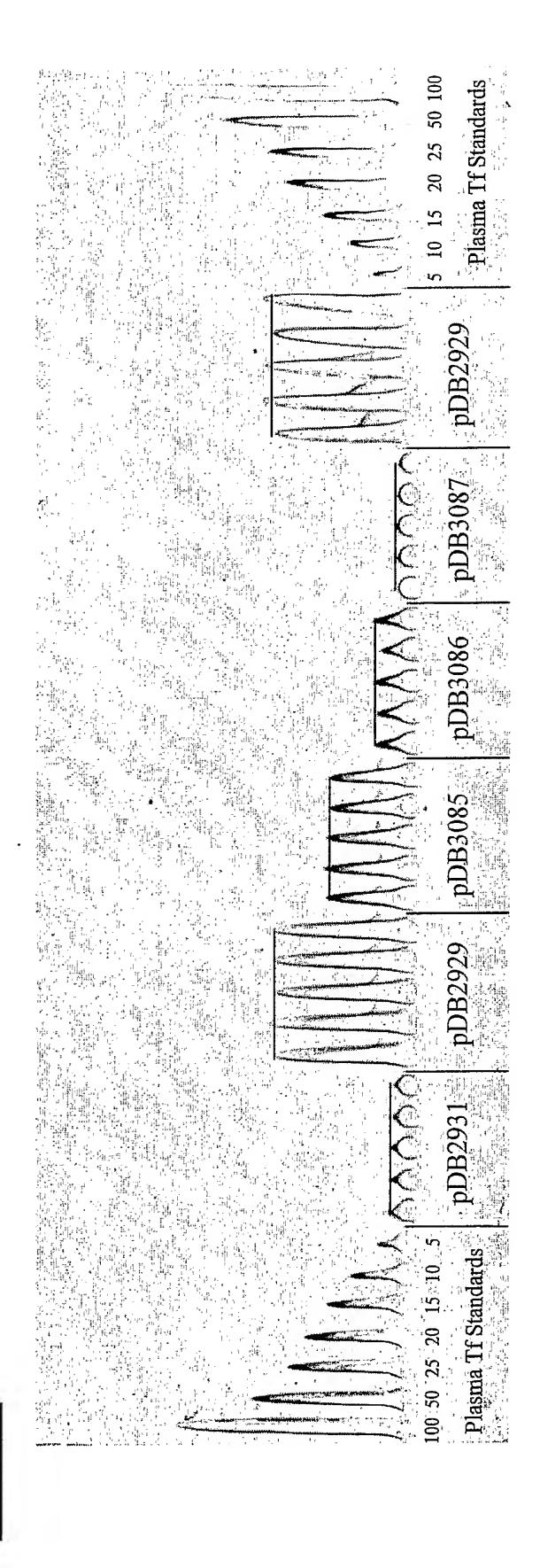




Xbal HindIII Xcm! Pst! Xcm! Xcm1 /Xcm1 Xbal Xcml Hind⊪ 页 mADH1t AmpR -*Hind* rTf (N413Q, N611Q) 页 Smal Smal Pstl mHSA-pre FP pDB3086 16204 bps Notl PRB1p REP2 PDI1 (S288c) LEU2 员 Smal Sfill Fsell Pacl SnaBl Xcm HindIII / HindIII 品 $\overline{\mathbb{K}}$ Xcml/ SnaBl/ Sma1 Psfl Xbal HindIII Hin dIII

FIGURE

FIGURE 54



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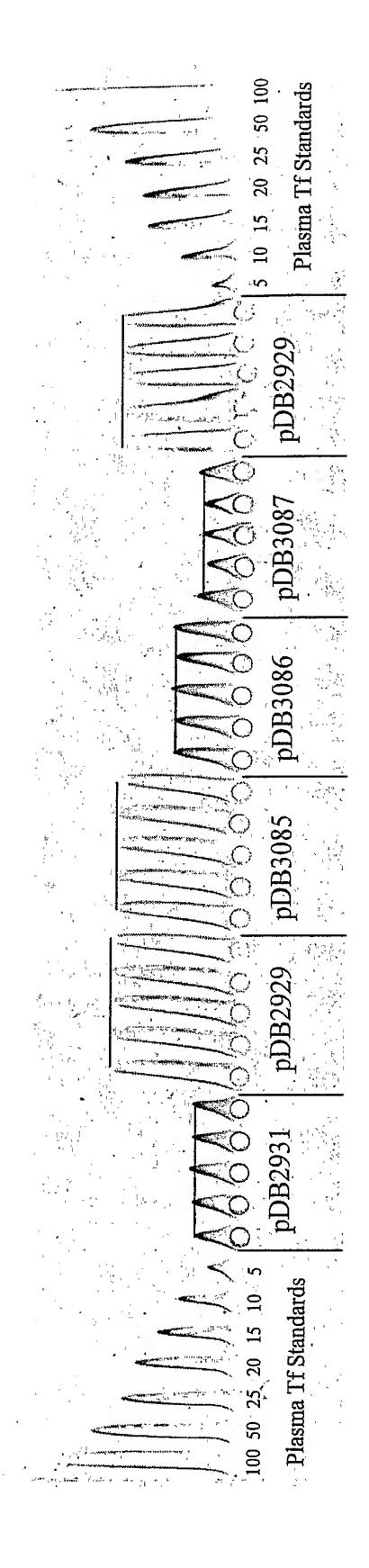
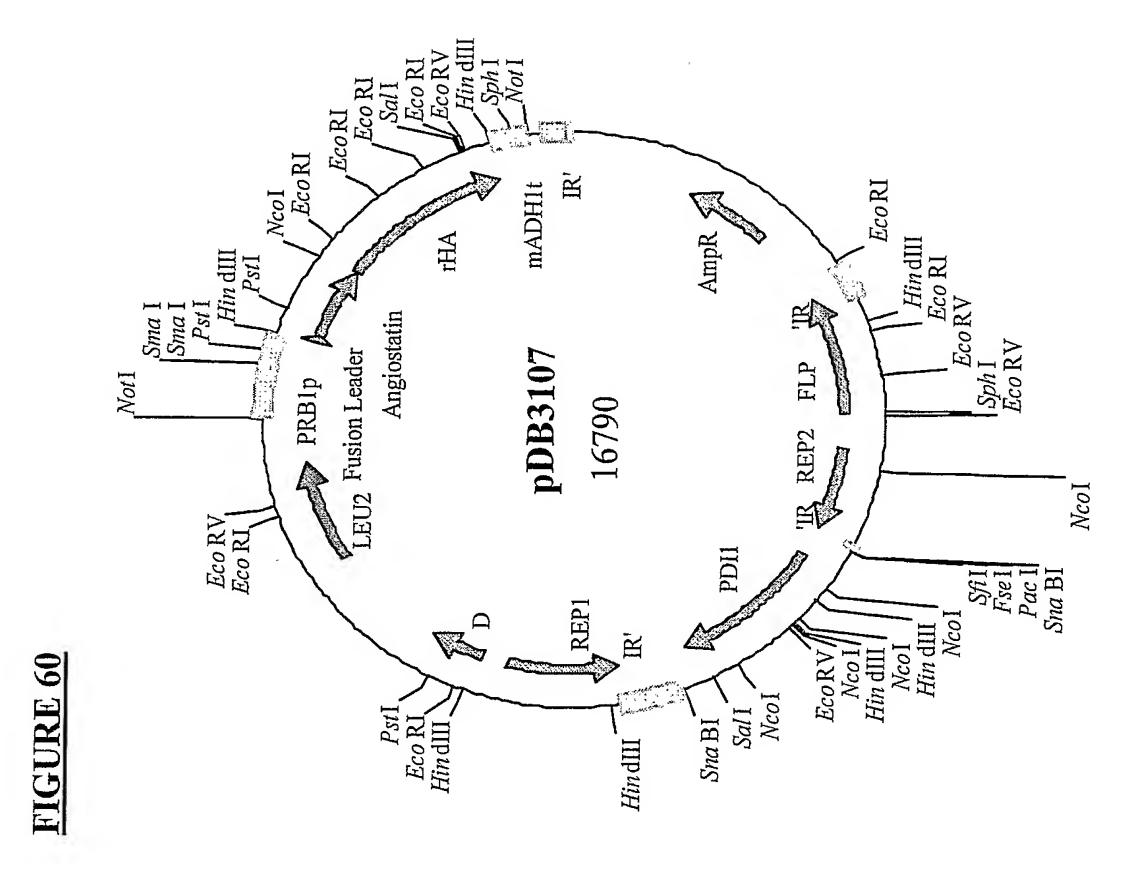


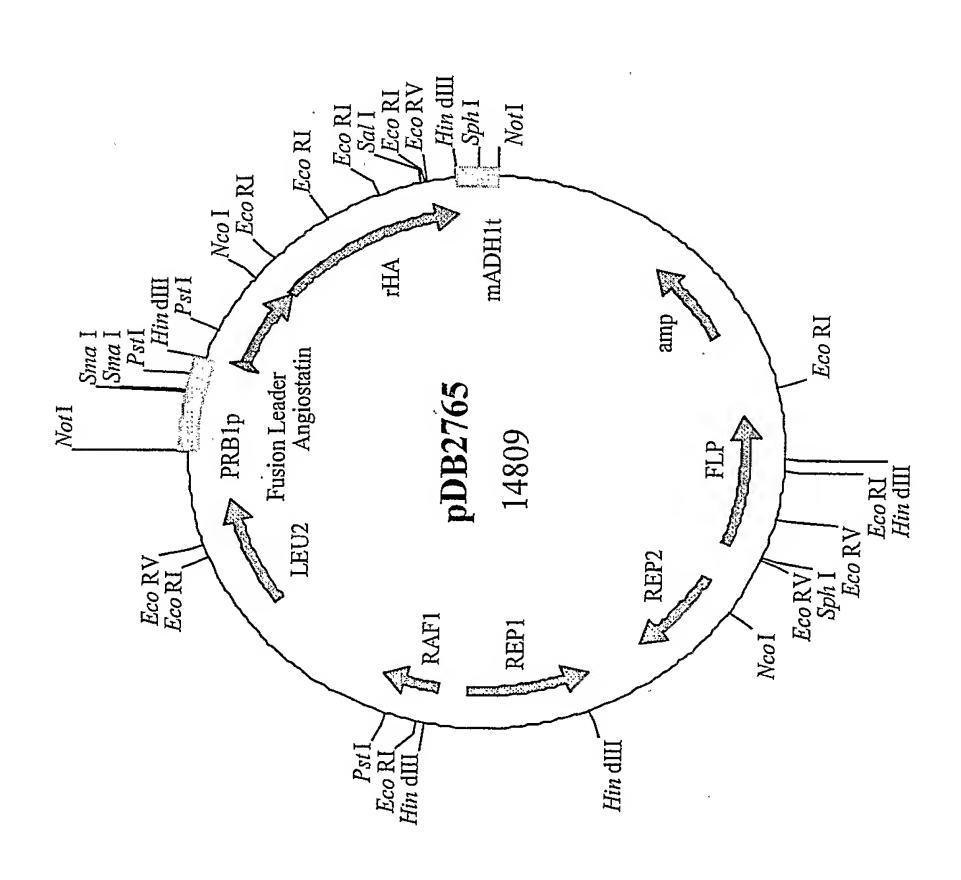
Figure 5

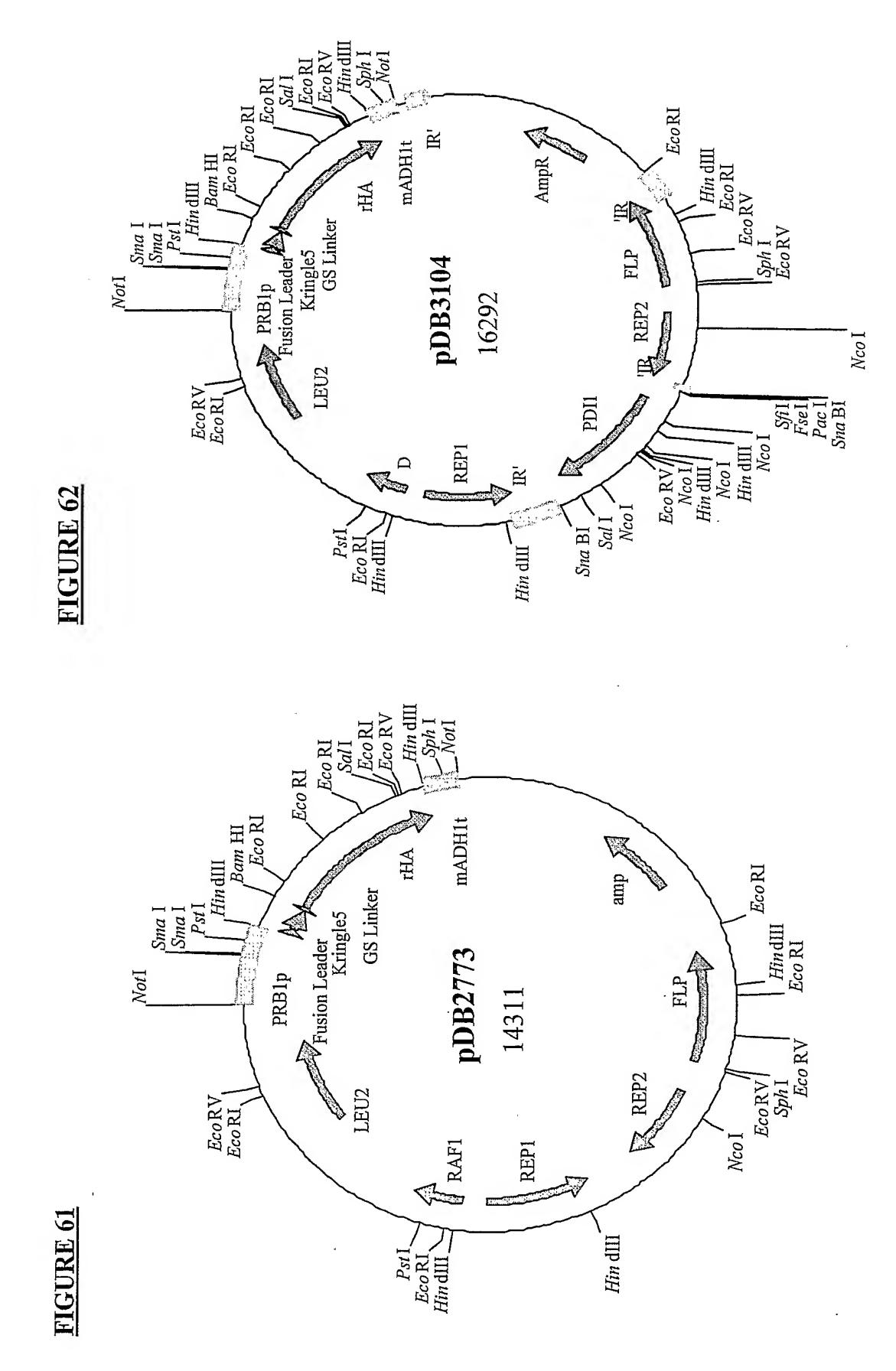
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58

Eco RI
Sal1
/Eco RI
Eco RI
Hin dIII
Sph I Eco RI Eco RI mADH1t IR' Sma I rHA Hin dIII Sph I Pst I AmpR Eco RI Sma I Sma I Endostatin Fusion Leader LEU2 Hin dIII pDB3099 FLP Noti PRB1p 14578 Eco RI $Eco\,\mathrm{RV}/|Sph\,\mathrm{I}|$ $Eco\,\mathrm{RV}/|Eco\,\mathrm{RV}|$ REP2 Eco RV | REP1 Ncol 田田 Hin dIII Pst I Eco RI Hin dIII







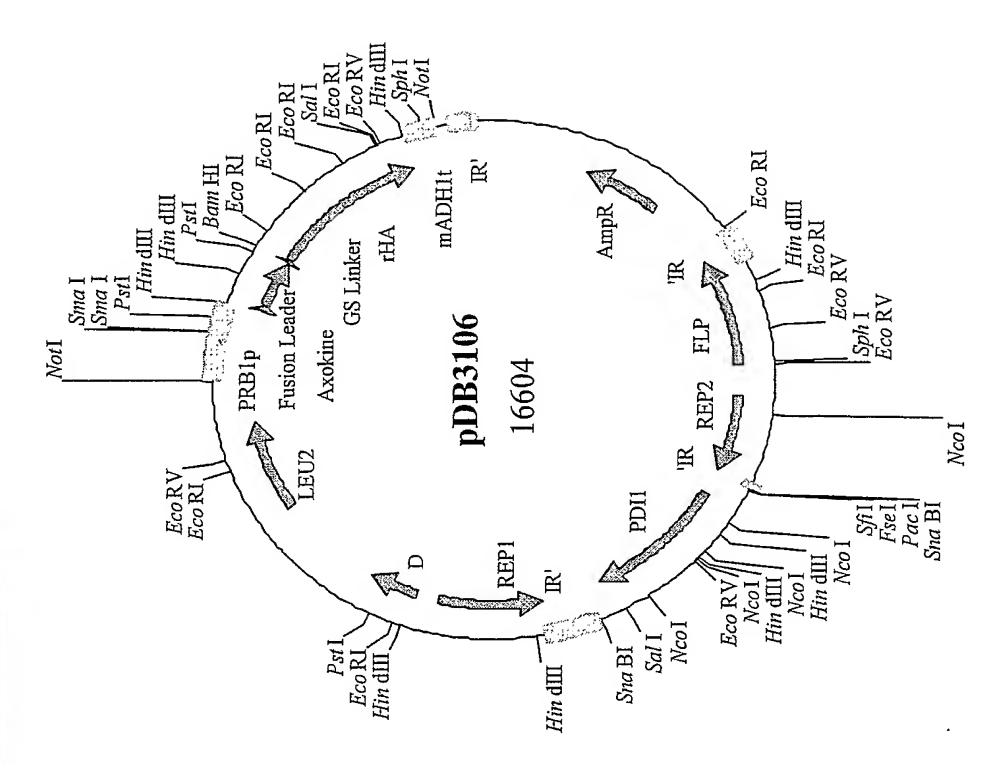
 $\operatorname{Hin}\operatorname{dIII}$ Vot Eco RI Sal 1 Eco RI EcoRI Eco RI AmpR 🎢 L HindIII
LECO RI
ECO RV
I 出 mADH1t Hin d III Bam HI Eco RIGS Linker Sph I Eco RV Sma I Sma I PstI Fusion Leader DX-890 FLP pDB3102 PRB1p NotI 16220 REP2 Ncol LEU2 Eco RV Eco RI PDII Sfill Fsell Pacl Sna BI REP1 Ω IR' NcolSall Sna BI Pst I Eco RI HindIII Hin dIII

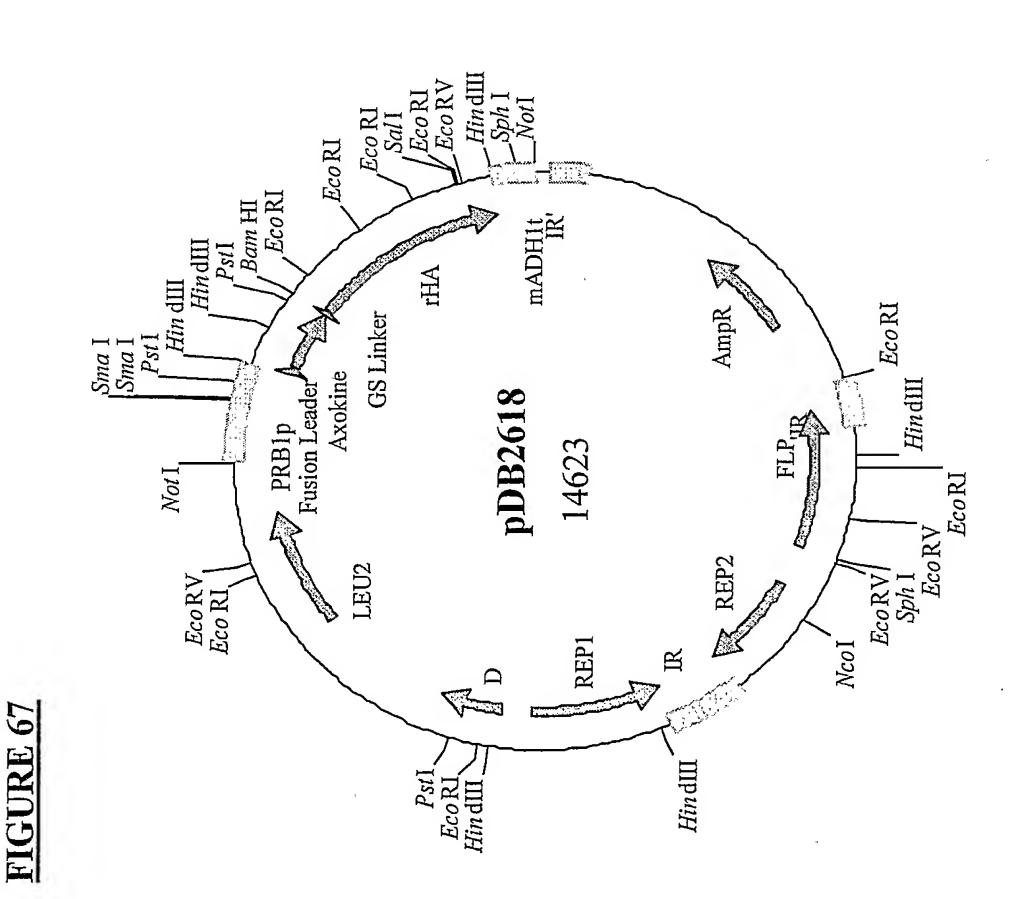
Hin dIII Sph I Not I Eco RI Sal I Eco RI Eco RV EcoRI IR' mADH1t Bam HI EcoRI AmpR 🚿 rHA Eco RI HindIII DX-890 GS Linker PRB1p Fusion Leader Sma I Sma I Pst I Hin dIII Eco RI 的 pDB3101 Not I 14239 FLP $egin{array}{c} Eco\, ext{RV} \ Sph\, ext{I} \ Eco\, ext{RV} \end{array}$ LEU2 REP2 Eco RV Eco RI Ncol REP1 果 HindIIIPst I. Eco RI HindIII

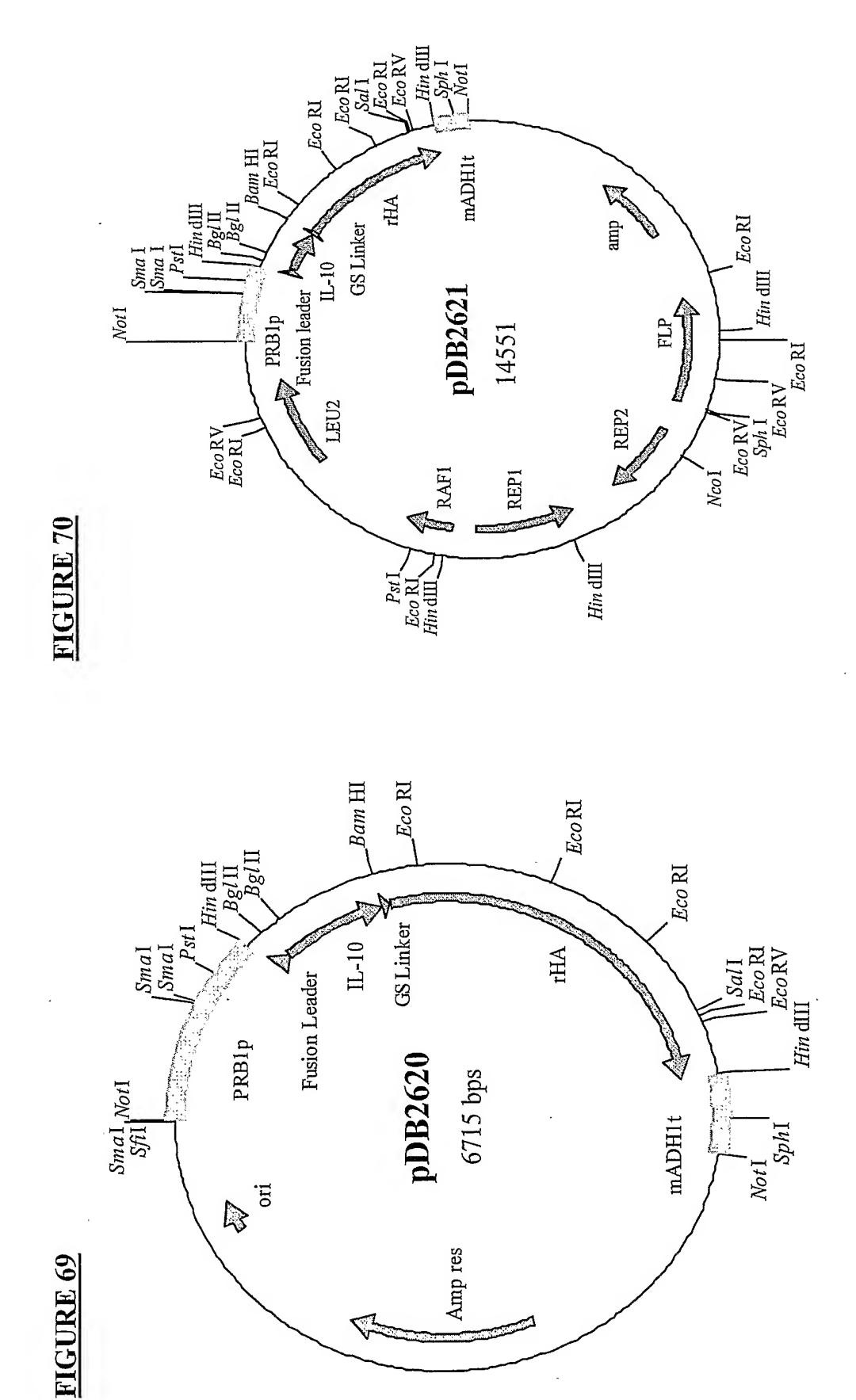
stI HindIII | Bam Hi | Eco RI | Eco RI Eco RI AmpR 6 EcoRV 民 mADHIt rHA Fusion Leader Fusion Leader DPI-14 GS Linker Sma I Sma I PstI FLP pDB3103 NotI 16232 REP2 Nco I LEU2 Eco RI Eco RI PDII Sfi 1 Fise 1 Pac 1 Sna B1 $Eco RV / \\ Nco I / \\ Nco I / \\ Nco I / \\ Hin dIII / \\ Nco I$ REP1 R NcoISall Sna BI PstI Eco RI HindIII Hin dIII

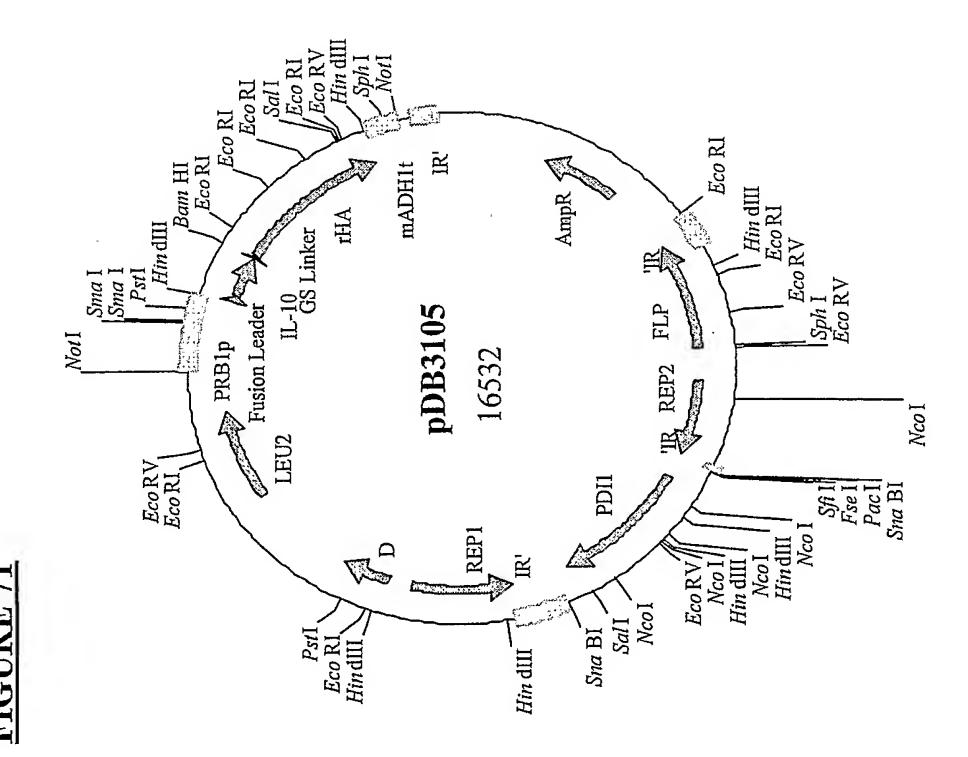
Hin dIII Sph I Not I Eco RI Sal I Eco RI Eco RV Eco RI mADH1t Eco RI Bam HI amp 📈 Eco RI HindIIIrHA **GS** Linker Sma I Sma I Pst I | Hin dIII Eco RI Fusion Leader DPI-14 pDB2679 Not I PRB1p 14251 FLP $egin{array}{c|c} EcoRV & Sph I \ EcoRV & \end{array}$ LEU2 REP2 Eco RI Ncol A RAFI REP1 HindIII Pst I. Eco RI. Hin dIII











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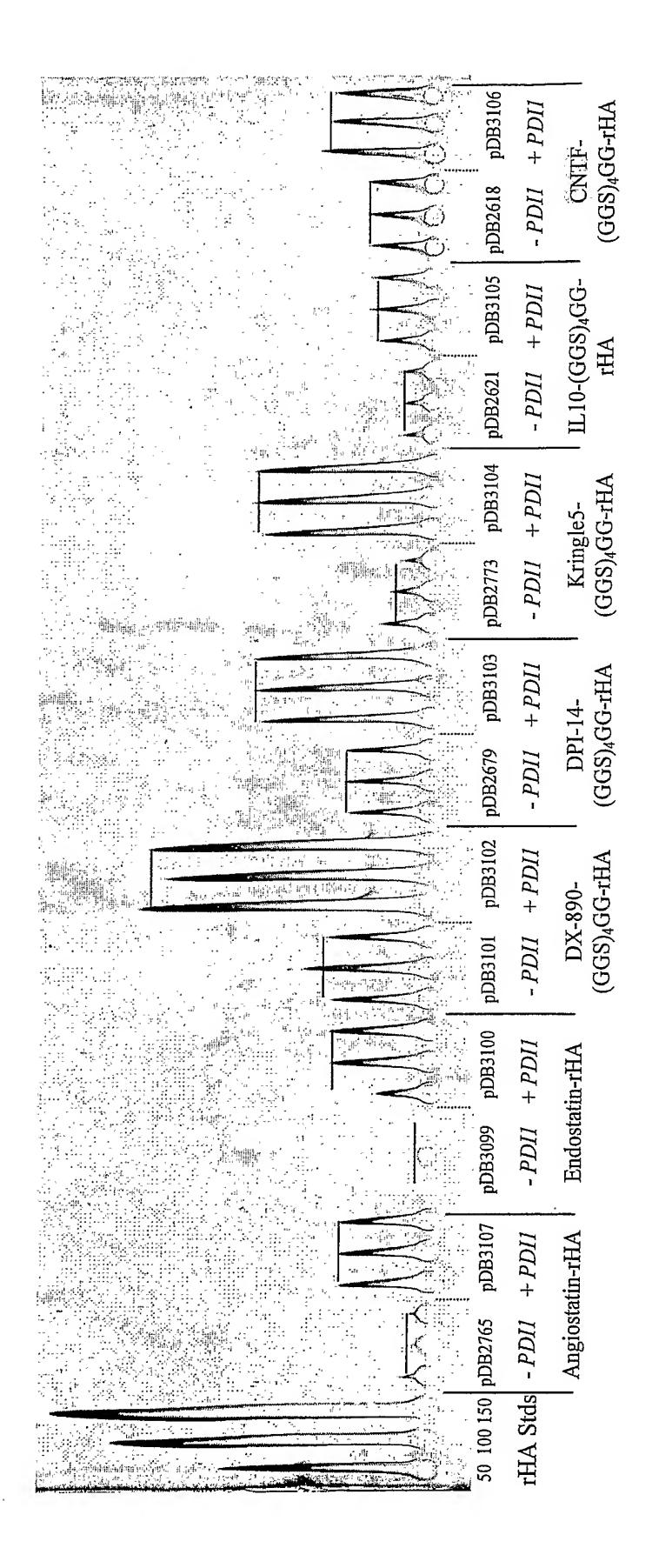


FIGURE 72

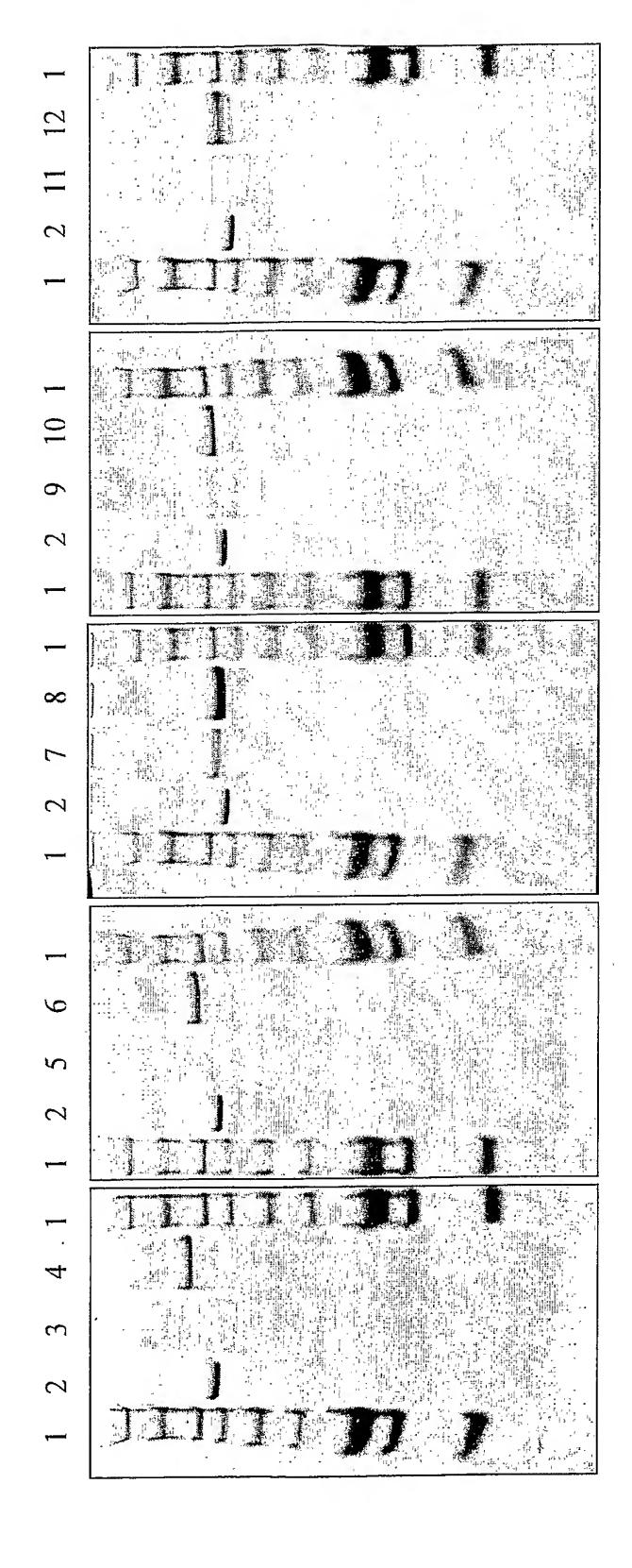
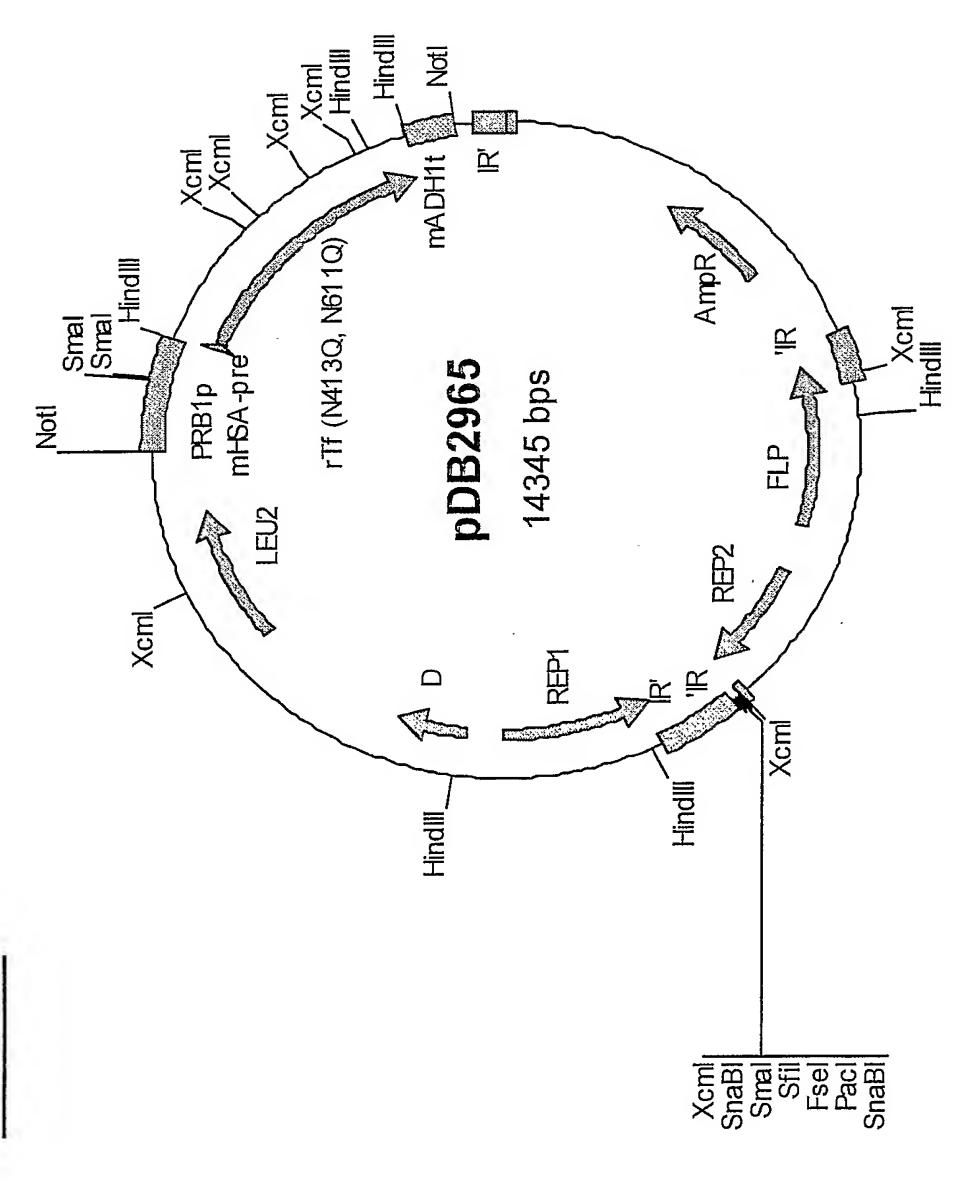


FIGURE 73

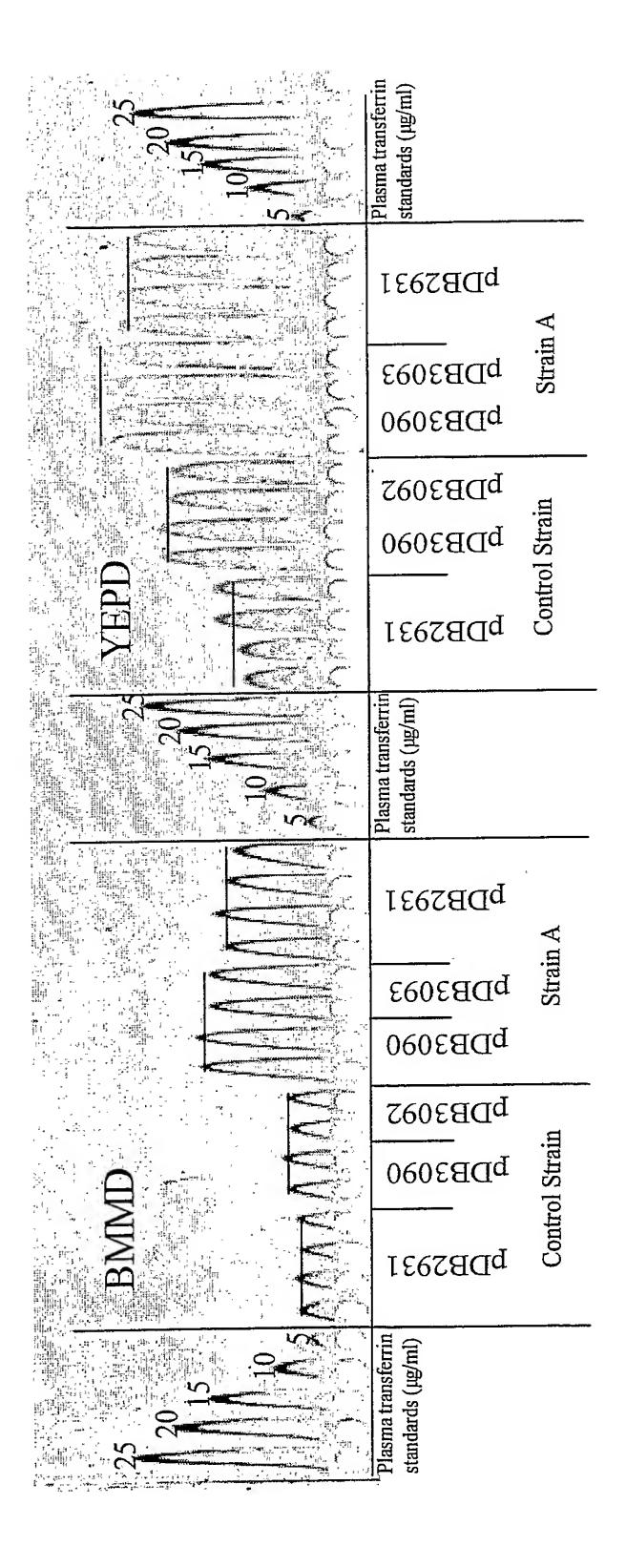


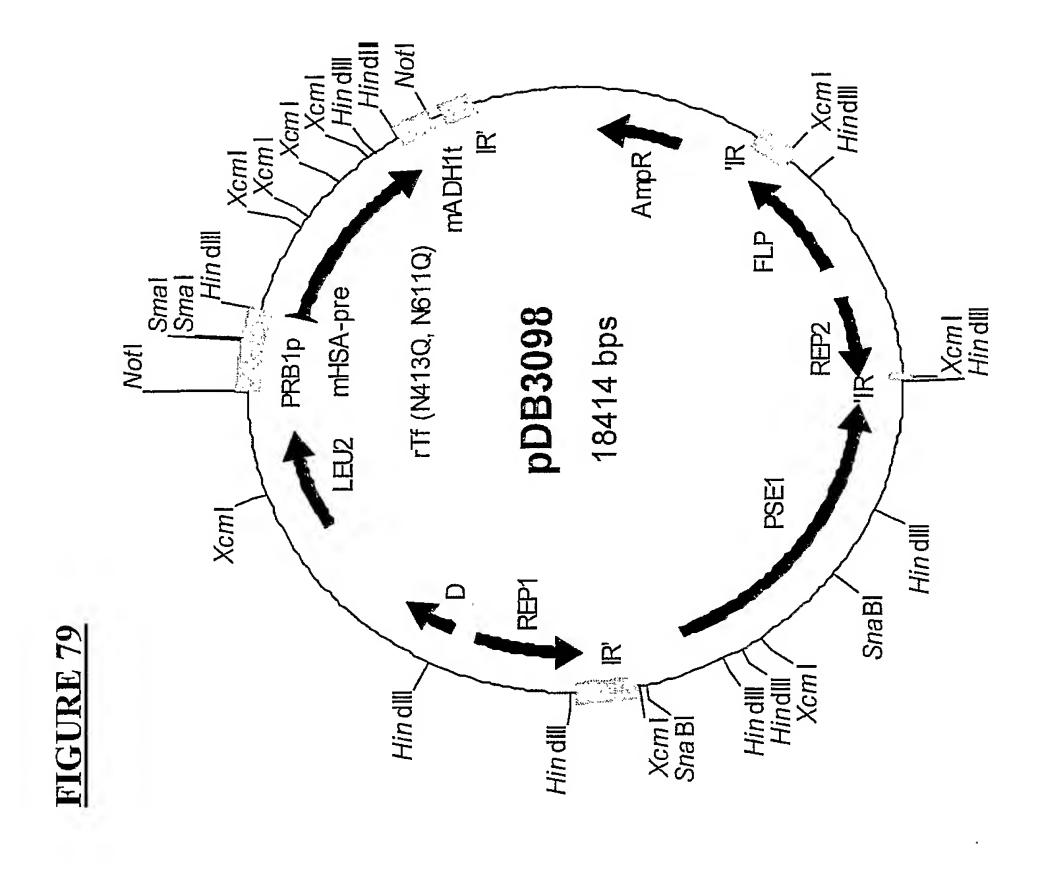
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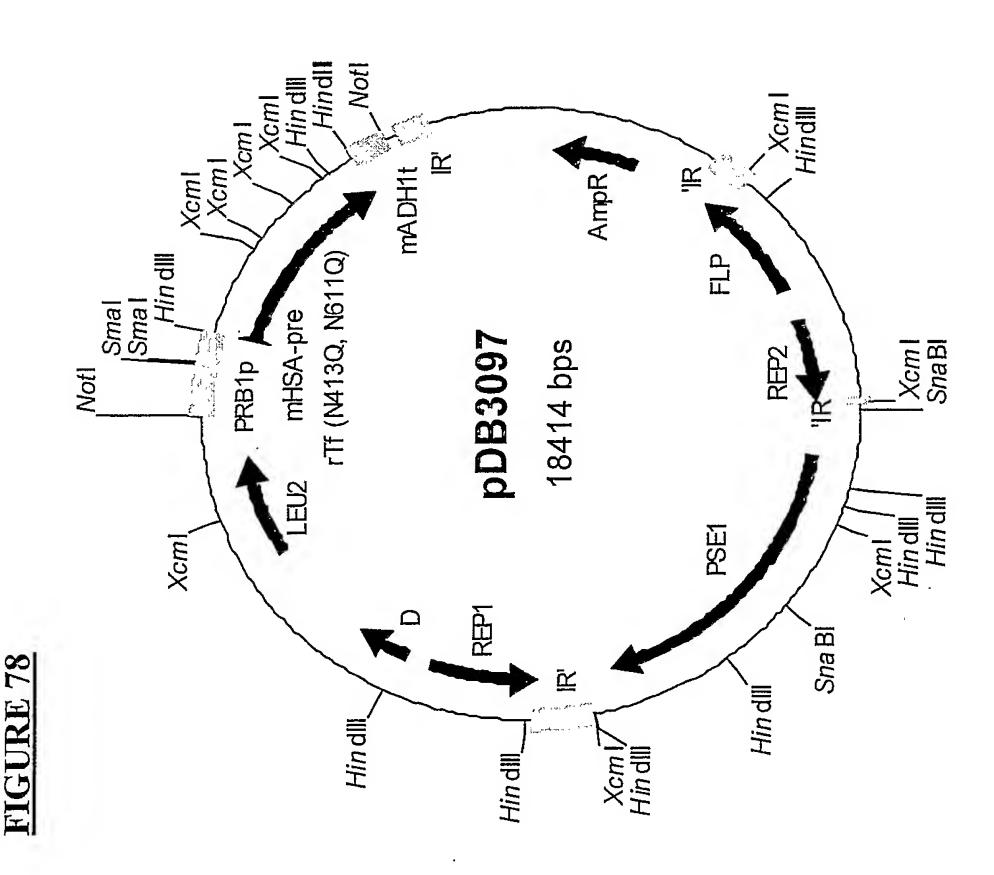
Hin dill Xcm Ndel Hind Xmn I Eco RI EcoRI Smal Bam HI PSE/ HindIII Ndel pDB2848 'lacZ' 6782 bps Xmnl amp Ndel Xmn | Sca | ✓ lacZ" ori Hindill/ Sph1/ Xba1/ Bam Hill

FIGURE 77

HindIII Not1 Xcml Xcml Hin dill Xcml Xcml <u>r</u> mADH1t AmpR : | Xcm| HindIII Hind pDB3090-pDB3093 员 rTf (N413Q, N611Q) Smal Smal mHSA-pre 15504 bps 딥 PRB1p Nof REP2 LEUZ ORMZ Xcm[SnaBl Pac I SnaBl Xcm I REPA 页 Xcm1/ Sna Bl Small Sfill Fsel Hin dill Hin dlll







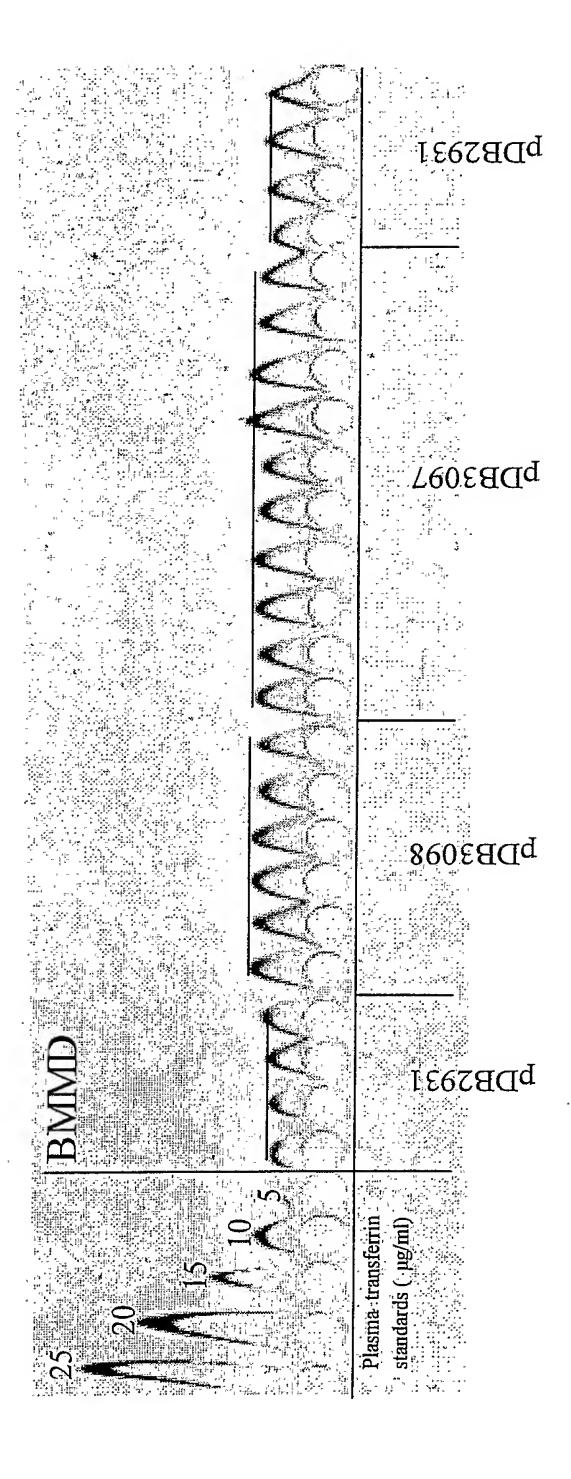
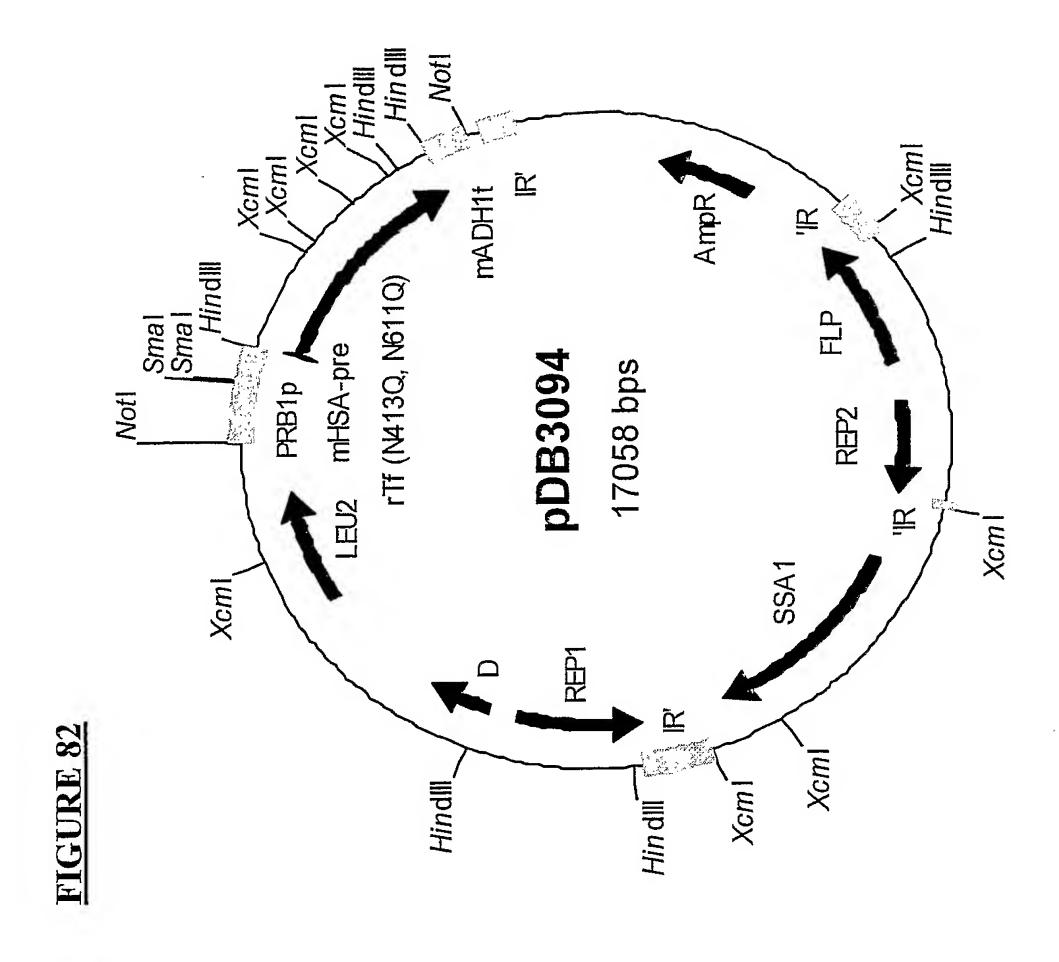
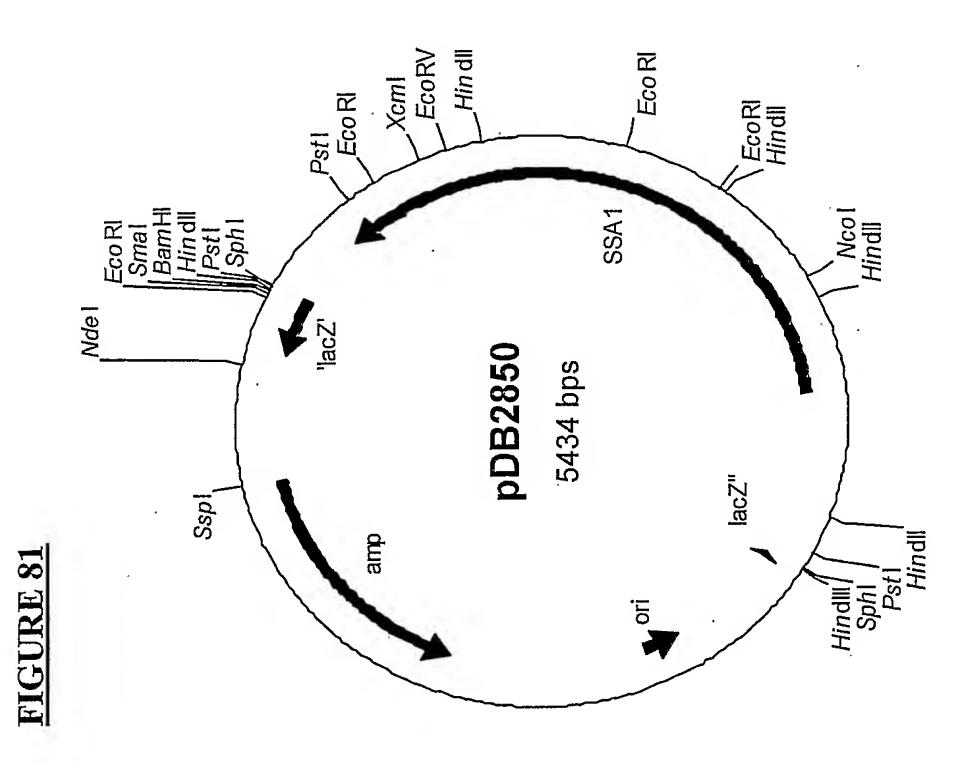
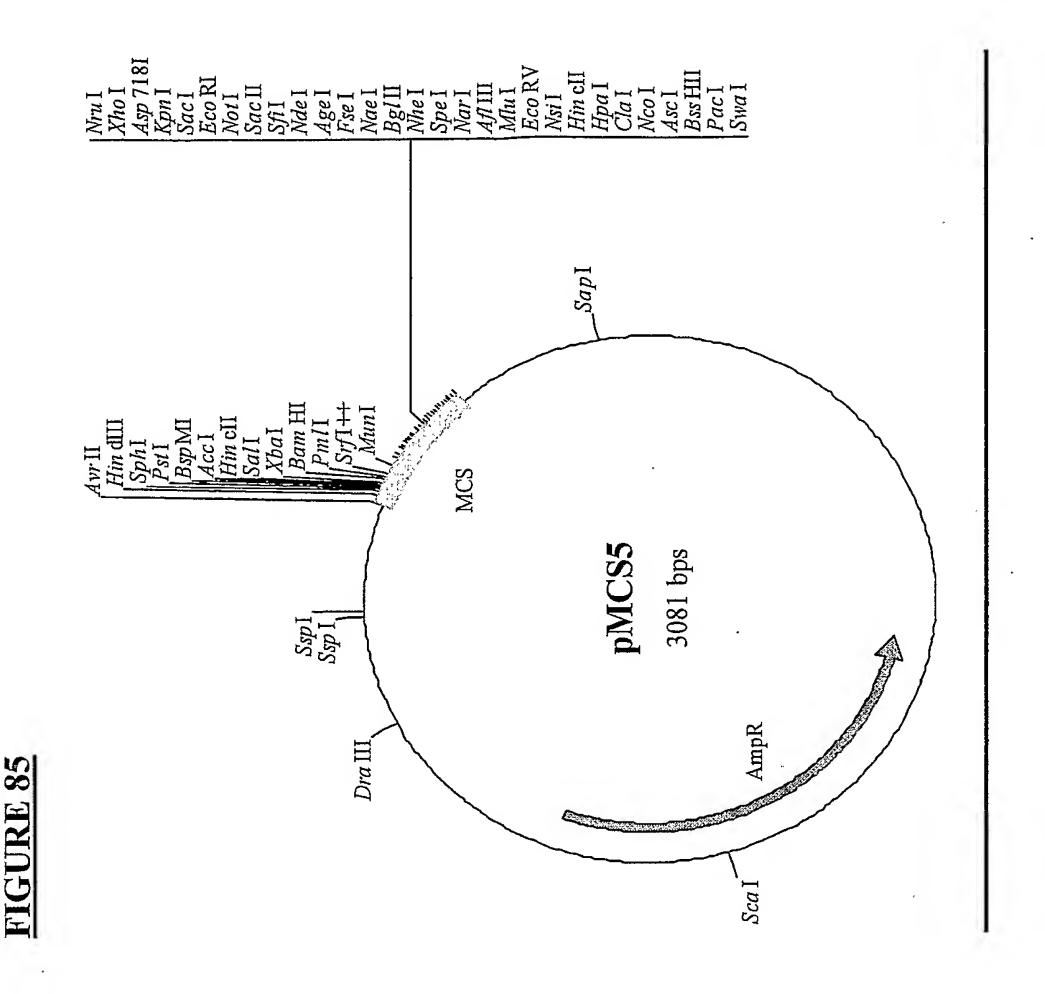


Figure 80







Xcml Xcml YHindll Hindlll , Xcml HindⅢ Xcml Xcml **mADH1t** AmpR 瓦 HindIII rTf (N413Q, N611Q) Smal Smal mHSA-pre pDB3095 17058 bps PRB1p Notl REPZ Xcm Xcml Xcml RECT HindIII Xcmi Hin dill

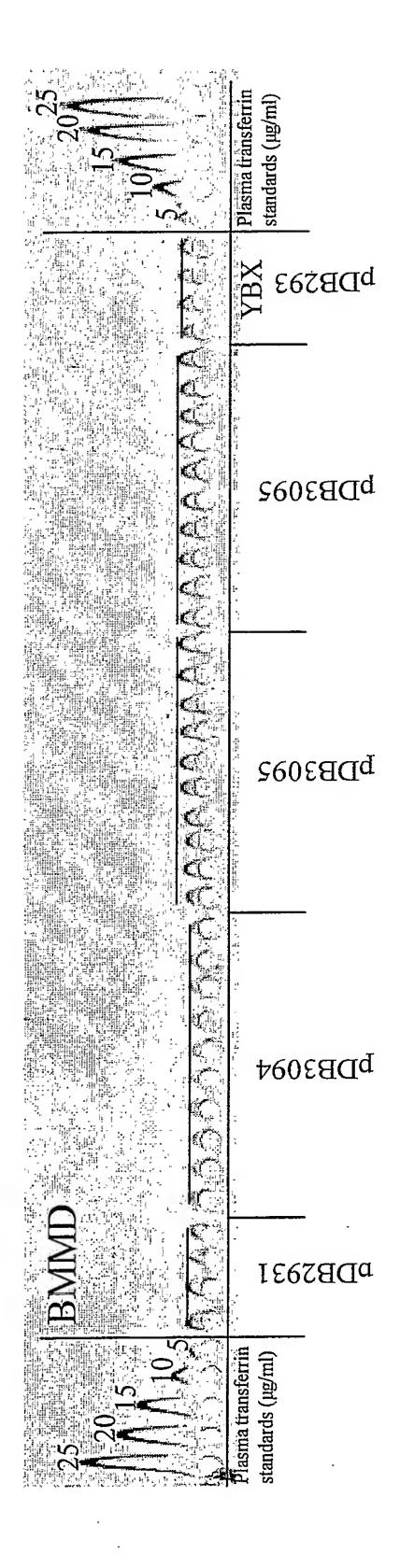
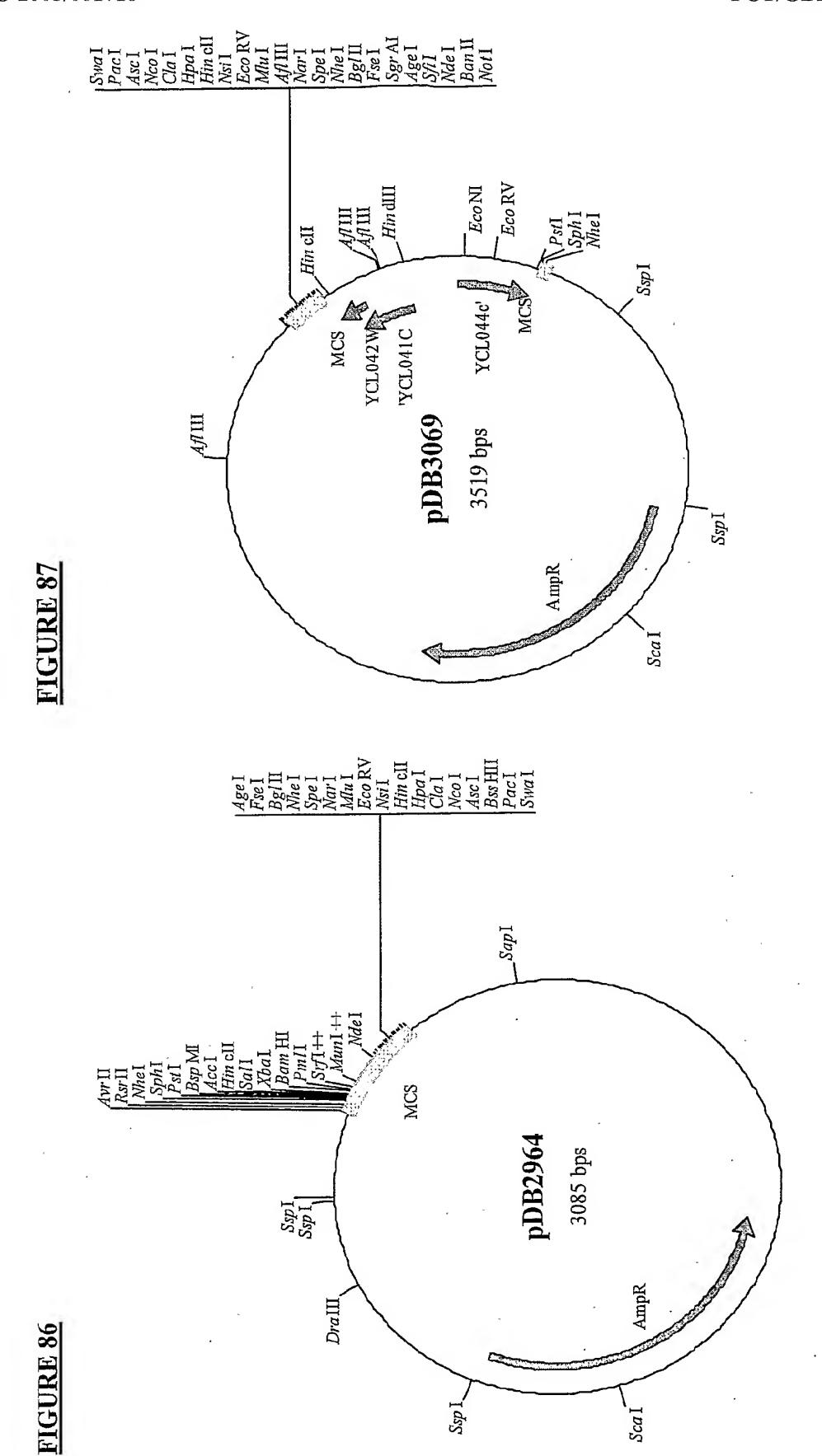
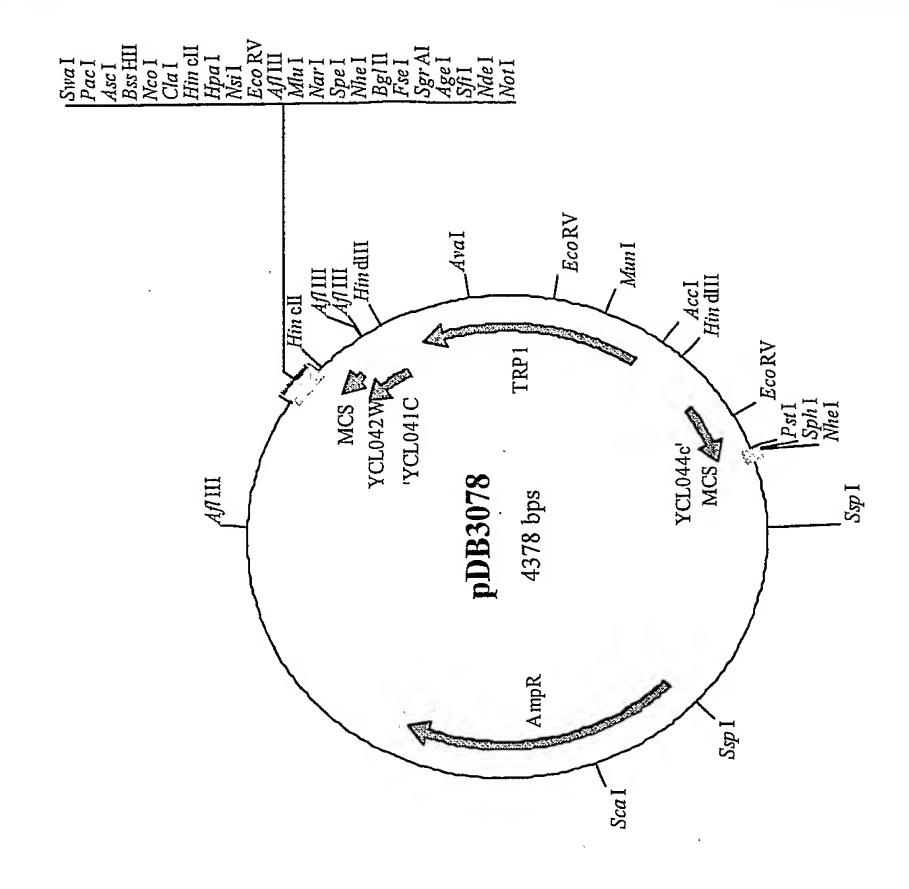
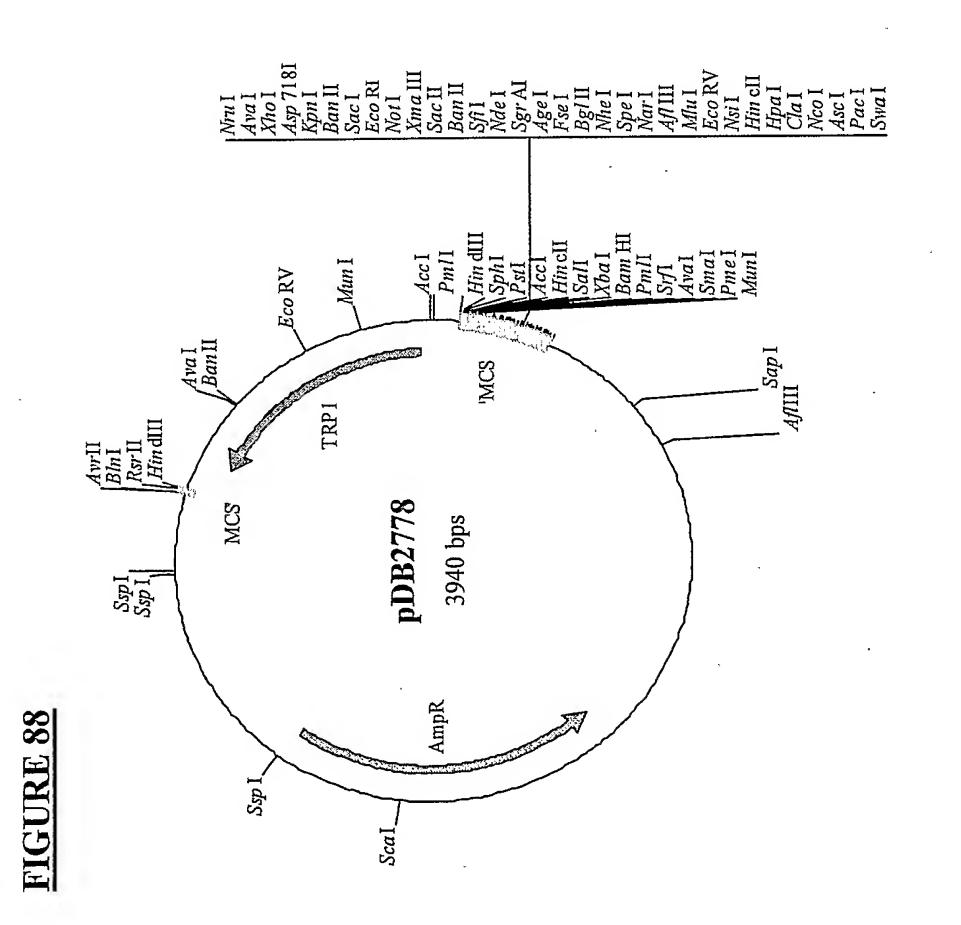
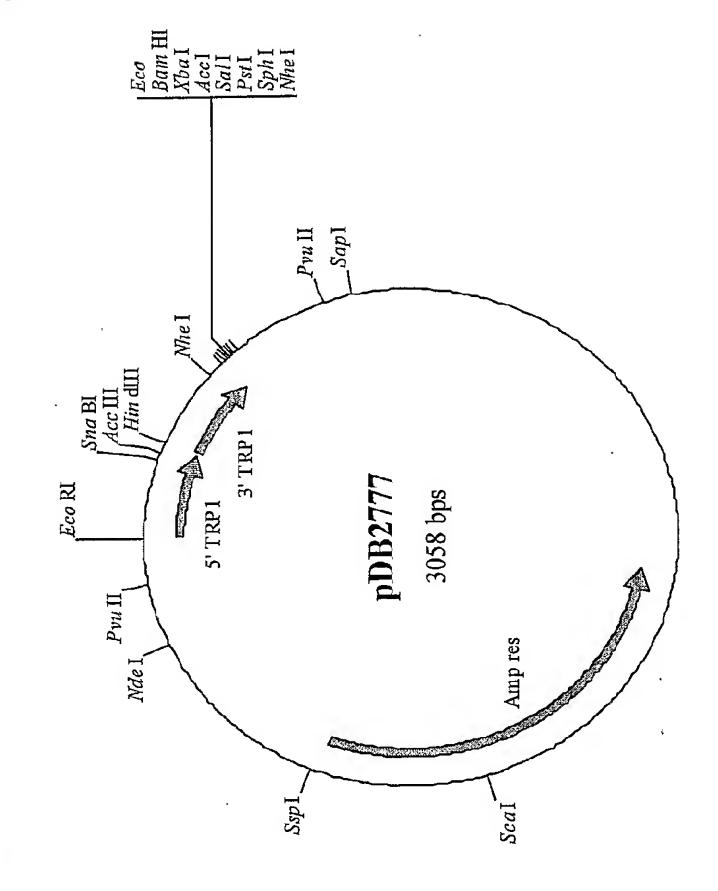


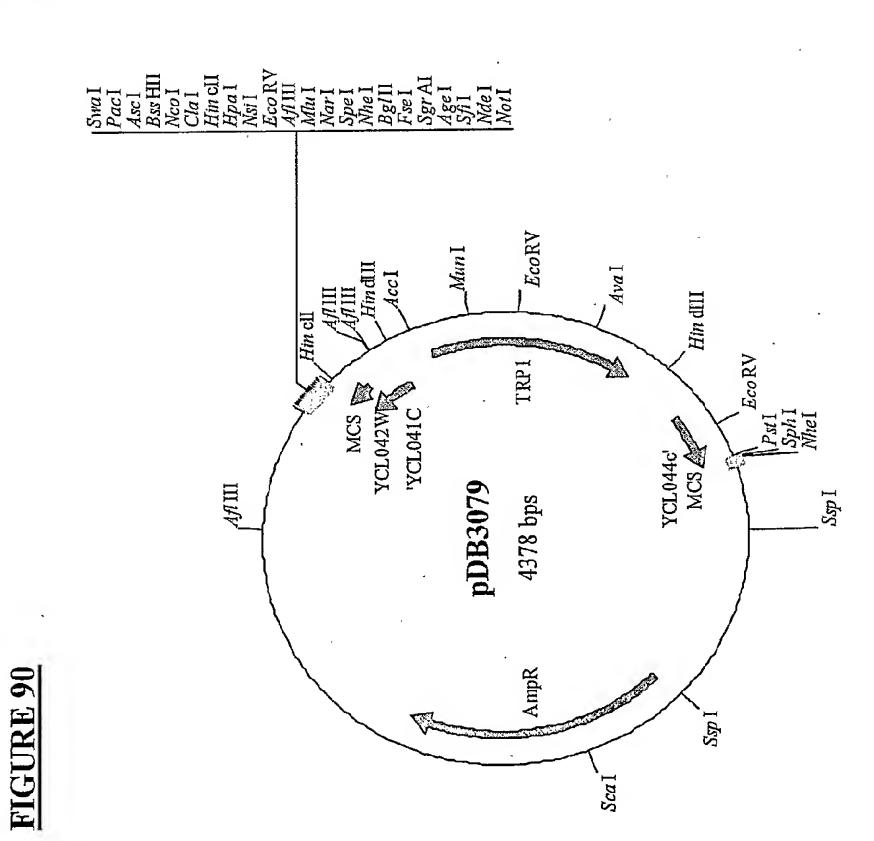
Figure 84











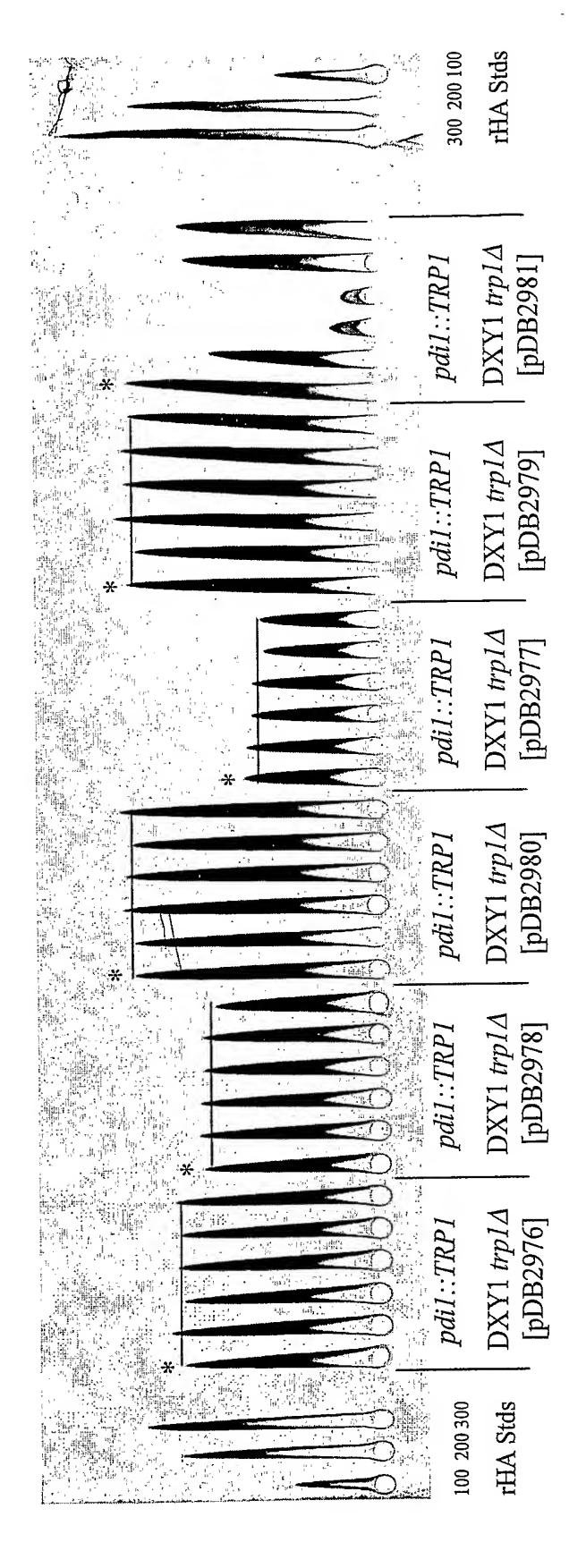


FIGURE 92

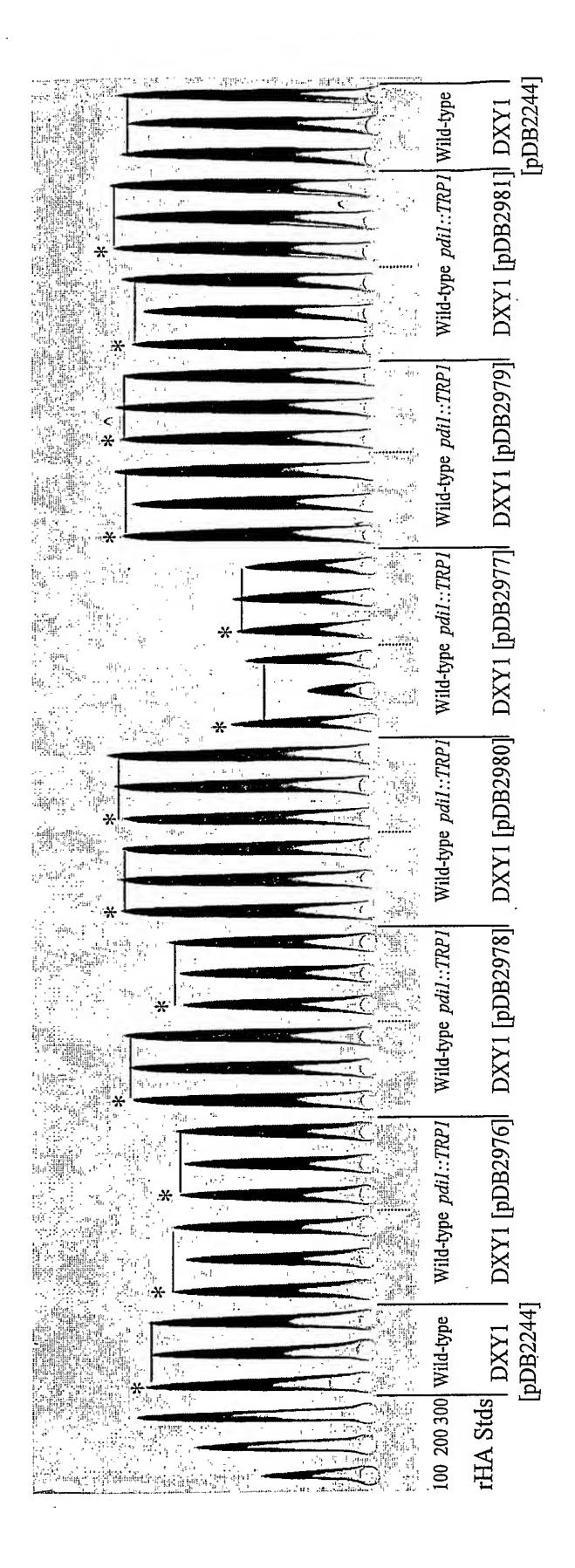


FIGURE 9

Page 1

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20 December 2004 15:04

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Figure 94B

Alignment Workspace of Ext.:meg J. Hein (Weighted) 20 December 2004 15:04

| ACCAGCITIACCI 670 ACCAGCITIACCI ACCAGCITIACCI | ACGACTACGACTITIGIC ICCGCTGAAACGCAGGATGTTTCAAGGTTTCTAGTTTACTITIGCCCTCCGGATGATTTTCAAGCTTTTGTCTTTGCCCTCCGGATGATTTTCAAGCTTTTTTTT | ATCOCTICACCTICANGITITICAAAAATGOTTICCAAGTGCAAGCCTTGCCCTACTTTTGGTGAAATCGCCCAATTTTTTTT | GGGTTPACTTIGETTCTPACAATGACGAGGAATTTGGAAGAATPACAAGCCTCTCTTTTPACCGAGTTTGGCCAAA 1000 1010 1020 1030 1040 1050 10 GGGTTPACTTATTCTPACAATGACGAAGAATTTGGAAGAATTACAAGCCTCTTTTTACCGAGTTTGGCCAA GGGTTPACTTGTTCTPACAATGACGAATTGGAAGAATTACAAGCCTCTTTTTACCGAGTTGGCCAA | CARARTICOGCACACOCCOGCAACIIGAACAIGAACAATICCCICTATITIGCCATCCACGACA 1110 1120 1130 1140 1150 1160 11 GAAAATICOGCAGCACOCGCAACIIGAACAIGAAGAACAATICCCICTATITIGCCATCCACGACA GAAAATICOGCAGCACCACCATCAACAIGAAGAACAATICCCICTATITIGCCATCCACGACA GAAAATICOGCAGCACCACCATCAAACAIGAAGAACAATICCCICTATITIGCCATCCACGACA | GROSCOTTICACCAATICAGCACAACAACAICTICAAGCIMITICAATCITICGITTAAGCACITICITICAAAGGICATGCCCCCAATCGICAAGTCCCAAGA 1220 1230 1240 1240 1250 1250 1250 1250 GROSCOTTICACCAATTGAGCCACAAGATCGIGITGGAGTCTATTGATTAAGGACTTCTTGAAAGGTGATGCCTCCCCAATCGIGAAGTCCAAGA 1310 GROSCOTTICACCAATTGAGCCACAAGATCGIGITGGAGTCTAAAGGTTTAAGGACTTCTTGAAAGGTGATGCCTCCCCAATCGTGAAGTCCAAGA 1320 | | | |
|---|--|---|--|---|--|--|--|--|
| S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | | | |

Figure 94C

| age 3 | | | | | | 2432 |
|--|--|---|---|---|--|--|
| Alignment Workspace of ExLimeg J. Hein (Weighted) 20 December 2004 15:04 | CGAAANCGICAACGACCCAAAGAAGGACGIICTIGITTITIGIR 1380 1390 1400 1410 CGAAANCGICAACGACCCAAAGAAGGACGIICITIGITTIGIR | GIRAGAGATTIGOCCCAACITACCAAGAACITACCIACGCCAACGCCATCCGACGITTTTGATTIGCTAAACITAGACCACTCGAAAACGATGTC 140 1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 GIRAGAGATTIGOCCCCAACITACCAAGAACTAGCTGATACCTACGCCAACGCCACCACCACCTCCGACGACTTTTGATTIGCTAAACTAGACCACTCGAAAACGATGTC 1530 GIRAGAGATTIGGCCCCAACTTACCAAGAACTAGCTGATACCTACGCCAACGCCACCACCACCTTTTTGATTIGCTAAAACTAGACCACTCGAAAACGATGTC 1540 | PANCHGINGIGIACCAAGGINCAAGANCCING 10 1620 1630 SAANCHGINGIGIACCAAGGINCAAGANCCING SAANCHGINGIGIACCAAGGINCAAGANCCING | TRAGOCCTTGTRACGRAPAGCCCAGGRAPAGCTGCTGRAGGCGAAGCTGACGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGAAGCCGGAAGCCTGAGGAAAAGCTGAGGAAAAGCTGAGGAAAAGCTGAGGAAAAGCTGAGGAAAGCCGAAGCCCGAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAACACACACCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCAAGCCCCCAAGCCCCAAGCCCCCAAGCCAACACCCCCAAGCCCCCAAGCCCCCC | ACCTGAATTOCTICACCAPACATCACCATTCACCATTCTCATTCACTTTTTTTT | CATABADAGATRAAATTCAAACACAATPGTTCGCTRATATTCGTCACCTCACACACACACACACACACACACACAC |
| ace of ExL. 4 15:04 | GAICTICGA 13 GAICTICGA GAICTICGA | GIGGICACII 14 GIGGICACII GIGGICACII | AGAGGCGTC 15 AGAGGCGTCC AGAGGCGTCC | CATCAAGGA 16 CATCAAGGA | ACGCTGAAT 1.7 ACGCTGAAT ACGCTGAAT | AAAAAAA-TCATTAAAAAAAA-TCATTAAAAAAAAATCATTAAAAAAAA |
| Alignment Workspace of 20 December 2004 15:04 | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long | S288c long SKQ2n long |

HindIII Sph I Noti Sall EcoRI EcoRV EcoRI EcoRI EcoRI HindIII PstI /BamHI mADH1t 黑' AmpR 🚿 HindIII EcoRI GS Linker PstI Smal Smal Fusion Leader Axokine HindIII IR. pDB2618 14623 bps PRB1p FLP í EcoRVI Sph1 i EcoRV EcoRI Notl LEU2 REP2. EcoRV EcoRL REP1 Ncol田 HindIIIPsd. EcoRI HindIII

Figure 96

BamHI EcorI. EcoRI HindIIIPsdHindIII EcoRI PstI Axokine $pDB2617 \ {\rm GS\ Linker}$ $egin{array}{c} | & SalI \\ EcoRI \\ EcoRV \\ HindIII \\ \end{array}$ Smal Smal Fusion Leader rHA mADH1t PRB1p 6787 bps Smal Noti Nod SphI ori B AmpR NdeI